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The Journal of Laboratory and Clinical Medicine

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CLINICAL AND EXPERIMENTAL

XANTHEMIA AND XANTHOSIS (CAROTINEMIA): A CLINICAL STUDY*

BY WILLIAM C. BOECK, PH.D., M.D., ROCHESTER, MINNESOTA, AND WALLACE M. YATER, M.D.,† WASHINGTON, D. C.

A DIET composed of much fruit, vegetables, butter or eggs, foods rich in yellow lipochrome pigments, may result in the development of a canary-yellow, or light orange pigmentation in the palms, soles, nasolabial folds, and sometimes, but to less intensity, in the skin generally. Von Noorden, in 1904, called attention to this condition among some of his diabetic patients and, in 1907, his observations were published. He thought it to be a manifestation of diabetes and named it "xanthosis diabetica." Following this announcement, other investigators recorded similar observations among diabetic patients. Moro, in 1908, discovered that the same type of pigmentation, "pseudoicterus," developed when carrots were added to the diet of some normal infants. This observation was recorded by other investigators later for both children and adults who did not have diabetes.

Salomon, in 1919, identified the pseudoicterus of nondiabetic patients as identical with the xanthosis diabetica of von Noorden. He and others showed that the pigmentation was of exogenous origin, and was derived by the absorption of the lipochrome pigments, carotin and xanthophyll, present in the fruit, vegetables, butter, and eggs of the diet. It disappeared in time when such foods were removed from the diet. Salomon pointed out that probably the first case of this nature, which he termed "xanthosis," was described by Hayem, in 1897, as occurring in an old dyspeptic patient. But according to Hashimoto, in 1922, Baeltz, in 1896, was the first to describe the pigmentation in persons

*Work done in the Division of Medicine, The Mayo Clinic.
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who had been eating many oranges for a long time. Van den Bergh and Snapper, in 1913, showed that there were lipochrome pigments circulating in the blood of the diabetic patients who had the yellowish discoloration of the palms, soles, and nasolabial folds. Spectroscopically it was found to be identical with pigments of carrots and other vegetables, chiefly carotin and xanthophyll.

Salomon, in 1919, also showed that these pigments were present not only in the blood of persons with the yellowish pigmentation of the skin, but in other persons who did not have discoloration of the skin. The circulation of the pigments in the blood he termed "xanthemia," and the condition in which the pigmentation occurred in the palms, soles, and nasolabial folds, "xanthosis," as von Noorden (1904) had done earlier. He found that xanthemia might occur without xanthosis but not vice versa.

Hess and Myers, in 1919, assigned the term "carotinemia" to those cases in which the yellowish discoloration of the skin occurred following the ingestion of a large quantity of vegetables over a long time. They adopted this term because they had failed to find any reference to this condition in the literature. However, the work of Moro, in 1908, and of Miura, in 1917, who had referred to the condition as "carotinosis," and the later work of Salomon, in 1919, had been overlooked. In view of this fact we believe the terms xanthosis and xanthemia have priority of usage, and that, also, they more accurately describe the conditions. Carotinemia literally means the presence of pigment circulating in the blood stream and does not imply the deposition of the pigment in the skin, as these authors intended. Investigations have shown that, although the chief yellow lipochrome pigment present in the blood serum is carotin, it is always associated with xanthophyll. On the other hand, Palmer, in 1922, cited a case of xanthosis in a patient with diabetes, whose blood serum, when analyzed for lipochrome pigments, contained mostly xanthophyll and but little carotin. His diet had been rich in xanthophyllic substances (eggs and green beans). The term xanthemia* is therefore more appropriate, and xanthosis should be used to signify the visible presence of these yellow pigments in the skin. We shall follow this usage of terms. A complete bibliography on this subject may be had by consulting the papers of van den Bergh and Muller, in 1920, Palmer, in 1922, Greene and Blackford, in 1926, and Stoner, in 1928.

Our investigations involved the study of 100 patients with diabetes, 53 patients with other diseases than diabetes, and 36 cases in which there was a diagnosis of xanthosis whether it occurred independently or associated with some disease.

We desired to ascertain in these patients the extent of xanthemia and xanthosis and to correlate these two conditions with such factors as sex, age, diet, disease, icterus-index and the serum-bilirubin content of the blood. We also made blood fat determinations in a small number of cases to see if there might be any disturbance of the fat metabolism of the body which might explain why xanthosis and xanthemia occurred in some patients and not in others.

*Strictly speaking the term xanthemia implies the presence of all yellow pigments circulating in the blood, but we wish to adhere to its original definition, as stated by Salomon, which limits the term to the lipochrome pigments, thereby excluding the bile pigments.

LABORATORY METHODS

A qualitative test for the determination of the lipochrome pigments in the blood was carried out one or more times for each case; a quantitative determination also was made in some of the cases. The specimens of blood were collected in the morning between eight and nine o'clock. The patients went without breakfast until after the collection was made.

Qualitatively, the presence of lipochrome pigments was demonstrated in the blood serum by adding 2 c.c. of alcohol, and 2 c.c. of petroleum ether to 2 c.c. of the blood serum and then centrifuging the mixture (Greene and Blackford). The yellow pigment, because of its solubility, passes into the upper layer of petroleum-ether, while the lower layer of precipitated proteins appears colorless. The intensity of the yellow in the petroleum-ether varied according to the amount of lipochrome pigments in the blood. In some cases the color was but faintly yellow and in others moderately or markedly yellow.

In most cases, the serum-bilirubin and icterus-index were determined at the same time that the qualitative test for the lipochrome pigments was made. In the course of the investigation cases were found in which the serum-bilirubin was normal, the icterus-index, relatively high, and the test for carotin pigment was positive. In these cases the excess of pigment in the blood serum, which accounted for the high icterus-index, was assumed to be due to the lipochrome pigments, a point emphasized by Greene and Blackford in 1926. We showed this to be true by determining first the icterus-index of the untreated blood serum, and then determining the index of the fraction of the serum treated with petroleum-ether. The icterus-index is always higher than the latter value when xanthemia is present.

Quantitative methods of determining the lipochrome pigments have been used before by other investigators. Reasonably accurate colorimetric methods were described by Willstätter and Stoll, in 1913, and by van den Bergh and Muller, in 1920. By the use of such methods values were determined after comparison of the extracted pigment in petroleum-ether with a standard solution of potassium dichromate. Kohn, in 1923, devised a spectrophotometric method for the quantitative determination of these pigments, and Niehaus, in 1924, compared the blood serum colorimetrically against a standard solution of 2 per cent chromic acid. We decided, however, to determine only the relative amount of lipochrome pigments in the blood by colorimetric comparison of the yellow lipochrome pigment with the potassium dichromate standard (1:10,000 solution) which is used in the determination of the icterus-index. We extracted the yellow lipochrome pigments from the blood serum by means of petroleum-ether as practiced by van den Bergh and Muller. In short, we determined the color index of the serum due to other pigments than bile pigments, and expressed it quantitatively by a value which we designated the lipochrome index.

The directions which we followed in determining the lipochrome index were essentially as follows: To a measured amount of blood serum in a centrifuge tube, add equal amounts of 95 per cent alcohol and petroleum-ether, shake well, and centrifuge. Extract the pigments by the petroleum-ether. Transfer the petroleum-ether layer to a graduated tube or small cylinder. Repeat the extraction of the serum as before until the petroleum-ether layer is colorless, removing this layer each time after centrifugation and adding it to the petroleum-ether fraction previously removed. The petroleum-ether fraction, containing the pigments, is made up, with more petroleum-ether, to a volume which gives a good depth of color for reading against the potassium dichromate standard employed for estimating the bile index, as used by Meulengracht, in 1921, Stetten, in 1922, Bernheim, in 1926, and others. Read in the colorimeter and calculate as follows:

$$\frac{\text{Standard set in mm.}}{\text{Reading of unknown in mm.}} \times \frac{\text{final dilution of unknown}}{\text{amount of serum used}} = \text{lipochrome-index}$$

Example: Two c.c. of blood serum were used. Extraction was done and the volume was made up to 6 c.c. A reading of 10 was made against the potassium dichromate standard which was set at 15 mm. The calculation, therefore, is as follows:

$$\frac{15}{10} \times \frac{6}{2} = 4.5, \text{ or the lipochrome-index}$$

Because of its simplicity this quantitative method permits a comparison of the icterus-index with the lipochrome-index, and by this means one can obtain a fair idea of the proportion of bile pigments and lipochrome pigments that produce color in the blood serum. In some of our cases the icterus-index was omitted and only the lipochrome-index was determined when the serum-bilirubin content of the blood was within normal limits.

CLINICAL INVESTIGATION

The occurrence of xanthemia and xanthosis, as stated earlier in the paper, was first determined for a group of 100 diabetic patients, then for a group of 53 patients with other disease, and without diabetes, and finally for a group of 36 patients with xanthosis in whom the xanthosis occurred either independently or associated with some disease.

Patients With Diabetes.—Of the 100 diabetic patients, 56 were males and 44, females. Their ages varied from four to seventy-four years, and they were distributed among the different age groups as shown in Table I. Sixty-nine per cent of the patients were between forty and seventy years of age; only 12 were in the first two decades of life.

1. Xanthemia. In 86 cases, 86 per cent, xanthemia occurred; it was absent in 14 cases. Practically all of the patients in the first four decades had xanthemia (Table I).

The results of investigations already referred to in the introduction of this paper have proved that the lipochrome pigments of the blood and skin in man originate from the ingestion of vegetables, fruits, butter, and eggs, in which these pigments are present. Since diabetic patients as a class partake of much of these foods, it was not surprising to find xanthemia in 86 of the 100 diabetic patients.

TABLE I

PRESENCE OF XANTHEMIA AMONG DIABETIC PATIENTS ARRANGED ACCORDING TO AGE GROUPS BY DECADES

AGE, YEARS	TOTAL CASES	XANTHEMIA		
		POSITIVE	NEGATIVE	PER CENT POSITIVE
0 - 9	2	2		100
10 - 19	10	10		100
20 - 29	11	10	1	91
30 - 39	5	5		100
40 - 49	20	17	3	85
50 - 59	25	20	5	80
60 - 69	24	20	4	83
70 - 79	3	2	1	66
Total	100	86	14	86

On their entrance to the diabetic service in the hospital, 27 of the 100 diabetic patients had been getting a normal diet; in 52, there had been a qualitative restriction of carbohydrates, and 21 had been receiving weighed diabetic diets. In the cases of the 21 patients who were getting weighed diets, rich in vegetables, tests for xanthemia were positive in 19 (90 per cent) and negative in two. In the 52 cases in which there was qualitative restriction, tests for xanthemia were positive in 45 (86 per cent), and negative in 7 (14 per cent); whereas of the 27 patients who received an ordinary, normal home diet, 22

(82 per cent) gave tests positive for xanthemia and five (18 per cent) gave negative tests.

It is certain that the patients who received a weighed diet for diabetes were eating more vegetables and butter than those who received a qualitatively restricted diet, and those, in turn, were eating somewhat more vegetables and butter than those who were getting the normal diet. Xanthemia would be expected to be increasingly more prevalent among patients in proportion to the amount of the pigment-containing foods they eat, and this is shown to be true in this investigation. The surprising thing was that there should be two patients on weighed diets for diabetes who did not have xanthemia.

There were two patients who had been receiving a normal diet before coming to The Mayo Clinic, where their diabetes was discovered, and who did not have xanthemia on entrance to the hospital. In these two patients, however, xanthemia was discovered in tests after they had been receiving a diet for diabetes for twelve days and for three weeks respectively. The diet included from 600 to 800 gm. of fruits and vegetables, one or two eggs and 30 to 60 gm. of butter daily. There were two other patients who were known to have had diabetes, six months and nine months respectively, who had been getting practically normal diets; tests for xanthemia on these patients were negative on entrance to the hospital, but they became positive on diabetic diets at the end of five and ten days, respectively. Sixteen patients with diabetes did not have xanthemia, however, even though they had been on diets for diabetes, containing 600 to 800 gm. of vegetables and fruit, one or two eggs, and 30 to 60 gm. of butter, from one to three or more days at the time the lipochrome test was made. Some of these patients were re-examined later and still did not have xanthemia after being on a weighed diet for diabetes from six to thirty-two days.

Regarding the relation of the severity of the diabetes to xanthemia it was found that in the patients who had xanthemia cases varied from those which were mild, requiring only dietary management, to those requiring varying amounts of insulin. Some patients had had diabetes for as long as twenty years, others for only a brief period. Some were in a state of acidosis and coma on entrance to the hospital. A few had gangrene of the toes. The patients without xanthemia had had diabetes from just a few months to as long as twenty-two years. Two of these patients also had gangrene of one of the extremities. As a group these patients had mild or only moderately severe diabetes.

2. Xanthosis. In nine of the 100 diabetic patients who were examined xanthosis was observed. All of these 9 patients also were in the group of 86 with xanthemia. Xanthosis did not occur in any patient who did not have xanthemia. This is in accord with Salomon's observations. All of the 9 patients except one, in whom the xanthosis was faint, had moderately severe or severe diabetes and all required insulin except the one noted above. This observation is similar to that of Rabinowitch, in 1928. Two of these patients had xanthoma diabeticorum as well, and one of these had entered the hospital in coma.

None of these patients was obese, although it was in obese children that Klose, in 1919, thought xanthosis developed most readily. For this reason, Klose thought that xanthosis might be related to the fat metabolism of the body. With this idea in mind, blood-fat determinations were made on 18 patients with diabetes. The blood-fat determinations were made through the courtesy of Dr. Luden of The Mayo Clinic, according to her modification of the Bloor methods. In Table II are shown the values for cholesterol (Bloor II), fatty acids and total fats, as determined in these 18 cases, and also the normal values determined by the same methods.

Two patients (Cases 66 and 96) had definite lipemia and possessed high (Case 66) and extraordinarily high (Case 96) blood-fat values. These two patients also had xanthoma diabeticorum. Cholesterol values in the other 16 cases were quite within the normal range of variability, although in some there was a tendency for the cholesterol to become elevated. The fatty acids and total fats were generally high as might be expected in patients receiving diets relatively rich in fat. It should be noted, however, that while there were 17 cases of xanthemia among these 18 patients, xanthosis was present in only four. Two of these cases were in patients with lipemia, but in the other two cases (Cases 12 and 88) blood-fat values were similar to those of many of the cases without xanthosis. The data based on this small group of cases would, therefore, tend to indicate that the blood-fat values bear no definite relationship to xanthosis.

Table II also brings out the point that when the lipochrome-index is high as in Case 12, xanthosis is likely to occur, but it may also occur when the lipochrome-index is as low as 2.2, and it may be absent when the lipochrome-index is even higher, as in Case 94 in which it was 3.5. The lipochrome-index does not seem to bear any definite quantitative relationship to xanthosis and represents only a relative value for the amount of lipochrome pigments in the blood stream at the time the test is made.

TABLE II

BLOOD FATS IN PATIENTS WITH DIABETES

CASE	XANTHOSIS	LIPOCHROME-INDEX	MG. IN EACH 100 C.C. OF BLOOD		
			CHOLESTEROL, BLOOR II	FATTY ACIDS	TOTAL FAT
Normal values			115 to 180	100 to 130	200 to 300
9	–		128	222	350
10	–	1.7	128	244	372
11	–		108	158	266
12	+	5.0	145	320	465
26	–		108	222	330
66	+	2.2	307	555	862
67	–	2.8	157	317	474
83	–		65	180	245
84	–	2.2	157	202	359
85	–	3.2	175	202	377
86	–	0.5	157	317	474
87	–	2.8	193	370	563
88	+	2.7	89	139	228
90	–	1.5	177	268	445
93	–	2.6	108	158	266
94	–	3.5	118	171	289
96	+	4.8	954	8888	9842
97	–	1.7	134	180	314

3. Relationship of the bile and lipochrome pigments of the blood. In the whole group of patients with diabetes, the bilirubin content of the blood serum varied from less than 0.3 mg. to 2.4 mg. in each 100 c.c.* of blood. The serum-bilirubin was within normal limits (0.2 to 2.0 mg. in each 100 c.c.) in all cases with the exception of one. It averaged 0.76 mg. in each 100 c.c. for the entire group. The icterus-index varied from 3 to 13.5; in nine cases it was 10 or more and the average was 6.9 for the entire group. This is slightly above the upper limit of normal which is 5.

In 13 cases the serum-bilirubin, the icterus-index, and the lipochrome-index were determined at the same time. In all of the cases, the serum-bilirubin was within normal limits; the highest value was 1.3 mg., the icterus-index varied from 3 to 13.5, and the average was 7.3 for the group of 13 cases. The lipochrome-index varied from 0.5 to 5 and averaged 3.5 for the whole group.

The relationship of the serum-bilirubin, icterus-index and lipochrome-index to each other is shown in Table III.

TABLE III

RELATIONSHIPS OF SERUM-BILIRUBIN, ICTERUS-INDEX AND LIPOCHROME-INDEX IN PATIENTS WITH DIABETES

CASE	SERUM-BILIRUBIN, MG. IN EACH 100 C.C.	ICTERUS-INDEX	LIPOCHROME-INDEX	LIPOCHROME-INDEX CALCULATED IN PER CENT OF ICTERUS-INDEX
12	1.2	9.0	5.0	55
66	1.3	7.7	2.2	28
67	0.7	5.9	2.8	47
84	0.3	5.0	2.2	45
85	0.3	7.5	3.2	43
86	0.9	4.8	0.5	10
87	0.7	8.8	2.8	32
88	0.5	6.3	2.7	43
90	0.3	3.0	1.5	50
93	0.3	5.0	2.6	52
94	0.3	6.8	3.5	52
96		13.5	4.8	36
99	1.3	9.6	3.0	31
100	1.1	9.8	1.6	16

The significant point in Table III is that the lipochrome-index was from 10 to 55 per cent of the icterus-index value. It averaged 38 per cent for all the cases. It is important, therefore, when evaluating the icterus-index to realize that probably one-third to one-half of the color of the serum is due to the presence of lipochrome pigments.

To summarize: (1) 100 patients with diabetes were examined qualitatively for the presence of lipochrome pigments in the blood and skin, of which 86 had xanthemia and 9, xanthosis; (2) the lipochrome-index averaged about 38 per cent of the icterus-index, which indicates that much of the color of the blood serum can be attributed to the lipochrome pigments of exogenous origin; (3) the presence in the diet of foods containing these pigments was undoubtedly a

*The van den Bergh technic for determining serum-bilirubin quantitatively admittedly is inaccurate when the values are lower than 1 mg. for each 100 c.c. of blood. This is owing to the difficulty of accurate matching of colors in the colorimeter. The figures for serum-bilirubin in Table II probably possess an inaccuracy of not greater than + or − 20 per cent. The error involved does not affect the conclusions.

large factor in the production of xanthemia, but other factors must be involved too, since some patients on diets rich in vegetables, fruits, butter, and eggs failed to have xanthemia; this finding may be related to the individual variation in the factors which regulate the absorption, circulation, deposition in the skin, oxidation and excretion from the human body of the lipochrome pigments; (4) xanthosis did not occur in the absence of xanthemia, but xanthemia often occurred without xanthosis; (5) xanthosis occurred more often in the severe cases of diabetes; (6) xanthemia did not seem to be related to the amount of lipoids in the blood except perhaps in lipemic patients, who also had severe diabetes, and (7) there was no quantitative relationship between the height of lipochrome-index and the presence or absence of xanthosis.

Patients Without Diabetes.—A series of 53 patients without diabetes but with other illnesses was examined for evidence of xanthemia and xanthosis. There were 13 patients with disease of the liver or biliary tract; 22 with renal disease; 3 with diagnoses of chronic nervous exhaustion; 2 with renal glycosuria; 2 with Addison's disease; 2 with orthostatic albuminuria; 3 with arthritis; one with exophthalmic goiter; one with lymphosarcoma; one with carcinoma of the stomach; one with static edema; one with hemolytic jaundice, and one with chronic appendicitis. Thirteen were females and 40 were males. Their ages varied from sixteen to sixty-nine years. Forty-two were from thirty to sixty years of age. Of this group, 47 (88 per cent) had xanthemia, and xanthosis was present in three (5.5 per cent).

Of the 13 patients with disease of the liver or biliary tract, all were in the hospital, where a study was being made to determine the etiology of their condition. Eleven of them were jaundiced. The cause of the jaundice was either stone in the common duct, stricture of the common duct, hepatitis (catarrhal or epidemic), cirrhosis of the liver, or metastasis to the liver. The serum-bilirubin content of the blood varied from 1.1 to 25 mg. for each 100 c.c. Only two patients had a normal serum-bilirubin, and these were not jaundiced at the time the test for lipochrome pigments was made, although the van den Bergh reaction was direct in both as it was in the 11 jaundiced patients. Xanthemia was present in 9 cases (69 per cent) and absent in 4 (31 per cent). It was not present in two cases of obstruction of the common duct, in one case of catarrhal jaundice, or in one case of pelvic and abdominal carcinomatosis with metastasis to the liver. Xanthosis was not present in the two patients who were not jaundiced, and it could not be determined because of the icterus in the jaundiced patients. All the patients in this group had been receiving normal diets before coming to the hospital, with the exception of one who had eliminated fats from the diet.

The group of 22 with renal disease was made up of patients with chronic nephritis, or with generalized or renal arteriosclerosis often associated with hypertension and myocardial degeneration. All of these patients had xanthemia, and two, xanthosis. One of the two patients with xanthosis had chronic glomerulonephritis, and the other, malignant hypertension with renal insufficiency. Sixteen of these patients had been receiving normal diets be-

fore coming to the hospital and only six had been getting diets for nephritis, low in proteins and probably high in vegetables and fruits. The incidence of xanthosis, 9 per cent (two cases), is identical with that among the diabetic patients examined. It occurred in one patient who had been getting a normal diet, which contained only an average amount of vegetables, and in another patient who had been eating much vegetable soup for four months.

There is still a group of miscellaneous cases in which, as in the previous two groups, diabetes was not concerned. In sixteen cases (89 per cent) there was xanthemia and in one case (11 per cent) xanthosis. There were two patients with renal glycosuria, both of whom had been on weighed diets for diabetes for some time. One had a serum-bilirubin of 0.7 mg. in each 100 c.c., an icterus-index of 9.7, without jaundice, and a lipochrome-index of 4.3 without xanthosis. This case is especially interesting because in some diabetic cases with a lower lipochrome-index there was xanthosis. In two cases each of Addison's disease, orthostatic albuminuria, and chronic arthritis, and in three of chronic nervous exhaustion, xanthemia without xanthosis was present. Likewise, xanthemia was found in one case each of exophthalmic goiter, carcinoma of the stomach, static edema, hemolytic jaundice, and chronic appendicitis. The patient with chronic appendicitis also had xanthosis; he had been on a high vegetable diet and the yellow color in the skin had been present for two weeks. In one case of chronic arthritis and one of lymphosarcoma tests for xanthemia were negative. All of these patients with the exception of the one with chronic appendicitis, had been getting normal diets before coming to the clinic.

In summarizing the results of the study of this group, composed of 53 cases of various diseases other than diabetes, we found that: (1) xanthemia was present in 47 cases (88 per cent); this figure is almost identical with that obtained in cases of diabetes (86 per cent); a normal diet had been given in 31 of these; (2) xanthosis occurred in only three cases (5.5 per cent), little more than half as frequently as in the cases of diabetes; (3) xanthemia occurred in the jaundiced patients about as frequently as in those who were not jaundiced; (4) patients with renal insufficiency due to nephritis, or associated with arteriosclerosis and hypertension, all had xanthemia even though the majority apparently were receiving normal diets; (5) two of the 22 patients with renal disease (9 per cent) had xanthosis and this incidence was similar to that found among diabetic patients, but the number examined was smaller; one of these patients with xanthosis had been on a normal diet, but the other one had eaten an excessive amount of vegetables for four months; (6) one patient with renal glycosuria who had been getting a weighed diet for diabetes for three months, did not have xanthosis, and the lipochrome-index was as high as 4.4 (serum-bilirubin 0.7 mg.); this was surprising, since some patients with diabetes and a lower lipochrome-index had xanthosis.

Thirty-six Patients With Xanthosis.—We reviewed 36 cases that had been diagnosed as xanthosis at The Mayo Clinic. There were 27 males and 9 females in the group. Their ages ranged from twelve to seventy years. Four patients were between ten and nineteen years of age; 4, between twenty and twenty-nine years; 9, between thirty and thirty-nine years; 12, between forty

and forty-nine years; 4, between fifty and fifty-nine years; 2, between sixty and sixty-nine years, and one was seventy years of age.

The chief complaints varied considerably: in ten cases it was diabetes; in twelve, stomach trouble; in five, weakness; in two, jaundice; and in one each it was one of the following: nervousness, vertigo, hypertension, asthma, goiter with throbbing in the neck, pain in the left lower quadrant, and constipation. The degree of xanthosis in these patients varied from definite yellowing of the palms, soles and nasolabial folds, to a more generalized yellow pigmentation of the skin.

It was thought advisable to learn the influence that this pigmentation had in determining the direction of the clinician's investigation. Naturally, the first supposition was that the color represented jaundice, and with this, the commoner possibility of cholecystitis. In this group of 36 patients, 10 had diabetes, and cholecystitis with jaundice was not considered a possibility in any of these, but 12 other patients complained of stomach trouble, biliousness or upper abdominal distress. Two of the latter group were sent to the clinic by their local physician with the diagnosis of suspected cholecystitis with jaundice. Six of these cases actually were investigated for cholecystitis at the clinic; but in the other 6, the diagnosis of xanthosis was made at once. In none of the 6 cases investigated for gall bladder disease was an ultimate diagnosis of cholecystitis made. It was apparent from the study of these 12 cases that when a patient with xanthosis complains of stomach trouble or upper abdominal disturbance, jaundice with cholecystitis often will be suspected by the clinician unfamiliar with the picture of xanthosis and probably will be investigated from that point of view.

The question of cholecystitis was raised, however, in three other patients who had not complained of upper abdominal distress. One patient complained of nervousness and another complained of pain in the left lower quadrant. Both of these had been on high vegetable diets for several months, eating many carrots each day. The ultimate primary diagnosis was hypochondriasis in the former case and only xanthosis in the latter case. The third patient complained of constipation. This patient was the only one in whom, ultimately, cholecystitis was diagnosed. The full and final diagnosis was neurosis, xanthosis and cholecystitis. The diagnosis of cholecystitis was based on the elicitation of slight tenderness in the right upper quadrant and an invisible gall bladder with the Graham-Cole technic. There was no history of gallstone colic. The serum-bilirubin had not been investigated and the scleroties were clear. The fact that the patient had been eating carrots daily for four months for her constipation readily explained the color of her skin, which was deepest in the palms and soles. There is only a question here, suggested by the roentgenogram, whether this patient actually had cholecystitis; clinically there was no evidence of it, except the slight tenderness in the upper quadrant, but even this may be partly discounted in view of the constipation and also the neurosis noted in the diagnosis.

Xanthosis was the sole diagnosis in four cases. The diagnosis of xanthosis was accompanied, however, by a final diagnosis of diabetes in ten, chronic nervous exhaustion in two, adenomatous goiter in one case, chronic appendi-

citis in two cases with operation in both, chronic glomerulonephritis in two, achylia gastrica in two, "functional stomach" in two, latent syphilis in one case, malignant hypertension in one, asthma in one, duodenal ulcer in one, cardiac neurosis in one, uterine myomas in two cases, chronic infectious arthritis in one case, neurosis and cholecystitis in one case and menopausal neurosis in one case.

The yellow discoloration of the skin also led the local physicians, in two cases, to suspect Addison's disease. One of these cases finally was diagnosed as exhaustion with hypotension and the other only as xanthosis. Two cases were investigated in the clinic for pernicious anemia but were later diagnosed as migraine and chronic glomerulonephritis. In another case, the question of chronic glomerulonephritis was raised and finally was diagnosed chronic glomerulonephritis and xanthosis.

The fact that 10 of the 36 patients had diabetes shows that this condition was more prevalent among such patients than among other persons who were ill or in good health. Even this number of patients with diabetes is smaller than the actual number, since the condition is so comparatively common in such patients that it is not always indexed.

The serum-bilirubin was investigated in 19 of the 36 cases. The highest value was 2.8 mg. and the lowest, 0.1 mg., with an average of 0.7 mg. The icterus-index was determined in 14 cases and varied from 3.0 to 15.0, with an average of 10.0. The high average (normal limits 1 to 5 inclusive) can be attributed to the extra color contributed to the blood serum by the presence of the lipochrome pigments in the blood stream, since the serum-bilirubin values were practically within normal limits. These data in the cases investigated, along with the clinical signs and symptoms, excluded the diagnosis of cholecystitis with jaundice, and afforded a valuable aid in making the diagnosis of xanthosis.

It is important to elicit as much information as possible concerning the diet of such patients, for the reason that the majority of those with xanthosis had been on a high vegetable diet for some time. Of the 36 patients with xanthosis, 31 had been on a high vegetable diet for various reasons. One patient had been on a diet for nephritis, one patient, who was ambulatory, had been getting a diet for peptic ulcer and one had been receiving a general diet. Information was not collected regarding the diet of two patients.

Eleven of the patients with xanthosis had been eating excessively of carrots, four, of eggs, and three, of spinach. Knowledge of the diet and the characteristic distribution of the pigment in the palms, soles, and nasolabial folds, but not in the sclerotics, are sufficient to identify the color as that of xanthosis and to rule out jaundice as a possibility. Should the sclerotics be icteric the possibility of both xanthosis and jaundice should be considered.

A summary of the study of the 36 cases of xanthosis* showed it to be

*Since the completion of this study two other cases of xanthosis have been observed. In one the diagnosis established was chronic nephrosis and in the other nervous exhaustion. Both of these patients had been receiving diets rich in lipochrome pigments; the patient with nervous exhaustion had eaten an average of 150 gm. of carrots daily for ten months and had had xanthosis for nine months. The other patient had been on a low protein, high vegetable diet for three years and had had xanthosis during the last eighteen months. These cases are remarkable for their high lipochrome-index; it was 12 in the patient with nervous exhaustion and 8 in the patient with nephrosis. The serum-bilirubin values were normal. The xanthosis of the patient with nervous exhaustion had been noted to vary from time to time in intensity.

due to dietary habits, and that the majority of the patients had eaten excessively of vegetables, especially of carrots, for a long time. Patients with diabetes, more than any other group of patients, had xanthosis. The yellow pigmentation of the skin often led to a suspicion of other conditions in which yellowish pigmentation occurs. There were no symptoms directly attributable to the xanthosis.

DISCUSSION

It is apparently established that xanthemia arises from the ingestion of foods rich in yellow lipochrome pigments and that probably it is a normal condition. Palmer (quoted by Head and Johnson, 1921) has stated that "carotin is unquestionably a normal constituent of human blood, the amount depending on the character of the diet." One would expect, however, a higher incidence of xanthemia among persons who eat a large quantity of vegetables for some special reason, than among persons who receive normal diets. That this is true is very well illustrated by our investigations among patients with diabetes and renal disease, as compared with the other types of patients. We have cited patients, nevertheless, who did not have xanthemia, even though they had been on a diet rich in lipochrome pigments for as long as a month. Why this should occur cannot be stated from the data gathered from this study. There is not sufficient evidence at present to attribute the phenomenon to any fault in the metabolism of fat. It seems probable that the pigments are absorbed from the intestinal tract because it is known that they become deposited to some extent in the adipose tissues to give them their yellow color. There seem to be individual variations among persons regarding the amount of lipochrome pigments circulating in the blood stream, and the length of time it remains there following the ingestion of these pigments. Also, it seems that there is individual variation in the rapidity of the disappearance of these pigments from the blood. The rate of oxidation of these pigments probably explains this variation, since, as Palmer, in 1922, showed, oxidation accounts for the normal disappearance of these pigments in both plants and animals.

The method described for the determination of the "lipochrome-index" is a simple one for quantitating the amount of the lipochrome pigments in the blood serum. It is easily comparable with the icterus-index, and, as we have found, the lipochrome-index may represent from 10 to over 50 per cent of the icterus-index. Bernheim, in 1926, made observations on four patients, each of whom possessed a normal icterus-index. Three of these were fed large amounts of carrots. The icterus-index became elevated but was normal again the next morning showing that the excess of lipochrome pigments had disappeared from the blood in twelve hours. Bernheim thought that this meant that all lipochrome pigments are removed from the blood within twelve hours. This assumption led her to believe that the high icterus-index which was found in many cases of diabetes was due entirely to an excess of serum-bilirubin and, therefore, indicated injury to the liver. But we have demonstrated that this is not true, since an appreciable fraction of the icterus-index is due to xan-

themia. However, the rapid disappearance of the lipochrome pigments from the blood (probably by oxidation) may explain why some of our patients on a diet containing large amounts of these pigments did not have xanthemia.

Investigations that have been conducted to determine whether lipochrome pigments have any pathologic effect when injected into guinea pigs and rabbits in relatively large amounts, have shown them to be harmless. Wells and Hedenburgh, in 1916, injected carotin intraperitoneally in guinea pigs and from their experiments concluded that "any such quantities (of carotin) as can ever accumulate in the tissues have no harmful effects." Connor, in 1928, found that when carotin dissolved in olive oil is injected intraperitoneally in guinea pigs, it becomes a foreign body and a characteristic tissue reaction occurs.

Xanthosis is the result of the deposition of the lipochrome pigments in the skin in such a concentration that the skin takes on a yellowish color. According to Palmer and Kempster, working with fowls, the lipochrome pigments are to be found chiefly in the rete malpighii and cornified layers of the skin. Miyake, in 1924, noted the same in the skin of man, and also found the pigment in the sebaceous glands. As Greene and Blackford pointed out, the deposition of the pigments in the layers mentioned, and the structures of the skin, account for the yellowness of the nasolabial folds where the skin is rich in sebaceous glands, and of the soles and palms where the cornified layer of the skin is thickest.

Xanthosis may, as we have seen, develop in some persons but not in others, although the amounts of vegetables consumed have been approximately the same. Hashimoto, in 1922, was of the opinion that "individual variation in the intensity of skin secretion or in the thickness of the epidermis, rather than the nutritional condition of the body, seemed to determine the tendency for the pigmentation of the skin of the subjects following a squash diet." There is, however, apparently a greater tendency for xanthosis to develop when diabetes is severe than when it is mild and the fact that it occurred as frequently in our series of the 22 patients with renal disease as in the group of patients with diabetes is suggestive that failure to excrete the pigments is probably an important factor in its production.

Three of the nine patients with diabetes, who also had xanthosis, were in a state of acidosis on entry to the hospital and another had been in coma one week before but was not in a state of acidosis on entrance. The skin of all showed marked yellowish pigmentation. Of the other five patients with xanthosis and diabetes, three had a severe grade of diabetes without acidosis, one had moderate diabetes and another had only a mild degree of diabetes. The xanthosis of these five, however, was not as marked as it was of the four with acidosis. One or more of several conditions may be responsible for this, such as imperfect excretion, dryness of the skin from dehydration, and disturbed oxidation in acidosis. In cases of xanthosis not associated with diabetes some of these factors may be operating.

Rabinowitch, in 1928, concluded that xanthosis in diabetic patients in-

dicates a poor prognosis. We cannot quite agree with this because: (1) the more severe the diabetes the more rigid as a rule is the diet and such diets are high in lipochrome content; (2) xanthosis did not influence the prognosis in 26 patients without diabetes, and (3) experimentally induced xanthosis is harmless.

SUMMARY AND CONCLUSIONS

1. The relative amount of lipochrome pigments contained in the blood plasma, a lipochrome-index, can be readily determined by the simple quantitative method described. Direct comparison with the icterus-index is possible and in many cases we have found the lipochrome pigments contributing from 10 to 50 per cent of the color of the blood plasma.

2. One hundred patients with diabetes and 53 patients suffering from other diseases were chosen at random and investigated for xanthemia and xanthosis. Xanthemia was found in 86 (86 per cent) of the patients with diabetes, in 22 (100 per cent) of patients with renal disease, in 13 (69 per cent) of patients with hepatic disease, and in 16 (89 per cent) of patients with other miscellaneous diseases. Xanthosis was present in 9 (9 per cent) of the patients with diabetes, in 2 (9 per cent) of the patients with renal disease and in one (3 per cent) of the patients with other diseases.

3. Among 36 patients whose condition was diagnosed as xanthosis were 10 with diabetes and 26 who had other diseases or, with the exception of the xanthosis, were normal. The majority of these patients had eaten excessively of foods rich in the lipochrome pigments, namely, vegetables, fruits, butter, and eggs.

4. The evidence does not warrant the conclusion that the presence of xanthosis as a complication of diabetes affects the course of the disease unfavorably. It is probable, however, that the ability of the individual to oxidize and excrete the lipochrome pigments varies and that there are some factors in some patients with diabetes that interfere with these processes; such factors may be acidosis and dehydration of the skin.

5. The yellow color of the skin in xanthosis may lead to confusion in diagnosis through its being mistaken for icterus. The distribution of the pigment in xanthosis is characteristic. The coloring is most intense in the palms, soles, and nasolabial folds, whereas the sclerotics usually are clear. A history of the patient's diet may be helpful in explaining the pigment, and determination of the lipochrome-index, icterus-index and serum-bilirubin permits of conclusive diagnosis. When yellow pigmentation of the skin is present and the serum-bilirubin is low and the icterus-index high, the condition must be xanthosis; if the serum-bilirubin is high and the lipochrome-index low, the abnormal coloration is due to jaundice; if both the serum-bilirubin and lipochrome-index are elevated, xanthosis is present, but is obscured by jaundice.*

*We regret that the paper by Stannus which appeared in the International Clinics for March, 1929 reached us too late for review. Any reference now will necessarily be inadequate because the paper is an excellent review of xanthosis. Stannus, like ourselves, concludes that xanthosis results from a failure to oxidize the carotinoid pigments in the body, although as we have stated this may be only one factor in the production of this condition. We do not favor the adoption of the term "hyperlipochromia," as Stannus suggests, to supplant the term xanthosis; our reason has been given in our paper.

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THE RÔLE OF STREPTOCOCCI IN THE RHEUMATIC DISEASES*

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IN ADDRESSING the Bath Conference on Rheumatic Diseases in May, 1928, Poynton[1] expressed the hope that "there will not be a splitting up of the study into heart, joint, and chorea units; for the constitutional disease and not the isolated lesion is still the main problem." This statement from so illustrious a pioneer in the study of rheumatic fever holds particular significance. It expresses that broad conception of the disease which is so essential if progress is to be made in elucidating its cause, or in combating it effectively.

Rheumatic fever should not be considered as an acute arthritis, as a disease of the heart, nor yet as a disease of any particular system or group of organs, but as a general disease producing characteristic pathologic lesions of widespread distribution. From the standpoint of the pathologist, it may be regarded as a disease producing lesions in endothelial structures wherever these are found in the organism. In a more restricted sense, it may be considered as a disease producing lesions in and adjacent to the blood vessels, and consequently its lesions occur in any tissue penetrated by even the smallest capillary.

From the clinical standpoint we have been too prone to think of the acute arthritis as the disease, rather than as an outstanding symptom of the disease. One may yet hear the cardiac manifestations spoken of as "complications" of rheumatic fever. This in a measure is excusable since from both the clinical and pathologic points of view, lesions of two types are recognized. These are designated the "exudative" lesions, best exemplified by the acute arthritis; and the "proliferative" lesions typified in their purest form in the subacute, progressive cardiac lesions. There is no doubt but that the former are promptly affected in a favorable manner by the administration of salicylates. Close students of the disease are in accord in believing that the latter type of lesion is not favorably affected by salicylates. Here then we have one of the enigmas of the disease, upon the solution of which hinges certain very puzzling questions of its etiology and pathogenesis. This situation at once suggests that the discovery of the agent will not clear up the subject, and that great difficulties would yet be encountered in determining the *modus operandi* by which the agent produces two types of lesions which differ so essentially in their characteristics as well as in their responses to a given form of therapy.

There is a third type of lesion which is likely to be overlooked. It is the "destructive" lesion. It is studied best in the heart muscle of patients who have died early in an attack of rheumatic fever, attended by an overwhelm-

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ing clinical toxemia. This lesion amounts to actual sterile necrosis of the muscle fibers, occurring in areas of a considerable extent.

The belief that rheumatic fever is an infectious disease has much in its support. The clinical manifestations of the acute attack suggest this. The seasonal occurrence of the disease is well known. The cyclic appearance of multiple cases in families has been emphasized by St. Lawrence,[2] and by Faulkner and White.[3] The association of the onset of the disease with attacks of acute tonsillitis, together with the many bacteriologic studies associating streptococci with the etiology of tonsillitis, has supported the connection between streptococci and rheumatic fever, as first emphasized by Poynton and Payne.[4] Atwater[5] recently has shown in epidemiologic analyses that the curves for the incidence of streptococcus infections and those for the prevalence of rheumatic fever run parallel in studies of the seasonal and geographic occurrences of these diseases.

Cultures of the blood in the acute attack of rheumatic fever have aided little, because it has been the common experience that these are in a large part sterile. Streptococci have been isolated perhaps more frequently than any other bacteria, but there has been no definite agreement in the types of streptococci as between the different workers, or as between the strains isolated by the individual worker. Yet, various as have been the strains isolated, experimental animals inoculated with them have developed acute arthritis. The different members of the streptococcus group, therefore, appear to have this feature in common, that they tend to produce arthritis in rabbits. It must be emphasized, however, that this experimental streptococcus arthritis has been for the most part a suppurative arthritis and quite different from the acute arthritis of rheumatic fever.

The exact reproduction of the microscopic lesion of the myocardium in rheumatic fever (nodule of Aschoff) has not been accomplished beyond dispute. Coombs,[6] de Vecchi,[7] Jackson[8] and Cecil[9] have produced myocardial lesions in rabbits closely resembling the nodules of Aschoff. Greatest attention has been given to the green producing streptococci in all of this work, but we do find some who have dealt with the hemolytic and with anhemolytic streptococci. Rosenow[10] in 1914 reported the isolation of three strains of streptococci which produced no effect upon the blood in the media upon which they were grown. He showed that these streptococci were capable of producing experimental lesions in animals.

In January, 1927,[11] I reported the isolation of an indifferent streptococcus in cases of rheumatic fever. It was obtained in the first instance from a blood culture, and later with great regularity from cultures of the pharynx in cases of rheumatic fever or of chorea. A large number of these strains have been collected. A recent report on the biologic and serologic relationships of 107 strains by Kreidler,[12] confirms my earlier observations on the compact nature of this group. They form the first compact serologic group of streptococci which has been associated with rheumatic fever, a fact alone which should stimulate considerable interest in this microorganism. The organism was named Streptococcus cardioarthritidis.

Further studies of this streptococcus,[13] demonstrated its intimate associa-

tion with the disease, rheumatic fever, its widespread geographic distribution, its ability to produce the tissue reactions and the lesions in rabbits and horses, which one is unable to distinguish from those of rheumatic fever, as has recently been emphasized by the work of Belk, Jodzis and Fendrick.[14] Its immune bodies (agglutinins and opsonins) are readily demonstrated in the sera of patients with rheumatic fever. Vaccines and soluble products of cultures of this streptococcus have been shown to precipitate acute exacerbations of rheumatic fever in individuals with low grade quiescent forms of the disease. Such exacerbations have been attended not only by acute arthritis, but by pericarditis, and the development of crops of subcutaneous nodules. The antiserum prepared from a single strain of this streptococcus has been employed over a period of two years in the treatment of patients, with results which have been more and more encouraging. The antiserum at first employed in relatively large volumes in an unconcentrated form, has later been concentrated by the globulin precipitation method until the volume of adequate dosage for the individual case has been reduced to 10 c.c. or less.

Closely following the original description of Streptococcus cardioarthritidis, Birkhaug[15] reported the association of nonhemolytic, nonmethemoglobin forming streptococci with rheumatic fever. From his description as well as from the reactions in the fermentation of carbohydrates, it is evident that his series contained strains of Streptococcus cardioarthritidis. This is further strengthened by his later work[28] in showing the effect of the antiserum of his strain (R. F. 24) in neutralizing the "primary" skin reactions of animals sensitized with Streptococcus cardioarthritidis.

Hitchcock[16] has also collected a number of strains of the anhemolytic streptococci, and has recorded studies which show that serologically specific species, other than Streptococcus cardioarthritidis, occur in this group. Unpublished studies by Seiger in our laboratory, have enabled him to identify serologically specific species of indifferent streptococci in addition to Streptococci cardioarthritidis. The studies of these workers show that Streptococcus cardioarthritidis is only one of a number of distinct species of streptococci which neither hemolyze blood, nor produce methemoglobin in artificial culture. They also emphasize the importance of the serologic tests in the identification of Streptococcus cardioarthritidis.

Lazarus Barlow[17] at the International Conference on Rheumatic Diseases at Bath, England, in May, 1928, presented a preliminary report on the neutralizing effects of antiserum upon culture filtrates of strains of indifferent streptococci isolated by himself, by Birkhaug and myself. From a study of the toxic effects of these filtrates on rabbits, his evidence indicated that a given antiserum, neutralized the filtrates of cultures of each of these strains with equal facility. This is an observation which if substantiated in further studies is important in fixing the identity of these strains isolated in widely different localities.

The studies presented in the recently issued Volume[18] of the Pickett-Thomson Research Laboratories stressed the identity of Streptococcus cardioarthritidis, and the strains supplied by Birkhaug and by L. Barlow, and states

that they are all closely related to Warren Crowe's Group A (Variety 3 or 4)[19] of anhemolytic streptococci.

H. Lowenberg,[20] of Berlin, has recently described independently a serologically specific streptococcus isolated from the pharyngeal cultures of rheumatic patients and of normal individuals. He designated this, pharyngokokkus. His report which includes the description of the cultural characters as well as a record of tests by agglutination and absorption of agglutinins of twenty-three strains of this streptococcus was prepared before the description of Streptococcus cardioarthritidis came to his attention. Upon the receipt of my strains he found that they conform both in cultural characters and in serologic reactions so that he concludes that there is no doubt of the identity of pharyngokokkus and Streptococcus cardioarthritidis.[21]

Since the work of Herry[22] in 1914, and particularly that of Faber[23] in 1915, there has been a growing tendency to explain the manifestations of rheumatic fever on the basis of bacterial allergy. The excellent and extensive work of Swift[24] and his coworkers has been important in developing facts in regard to this theory. In a late report Swift[25] definitely declared himself in favor of the allergic theory. Zinsser and Yu,[26] in a recent report also express this conception of the disease.

This conception differs essentially from those which attribute the disease to a bacteremia or a toxemia in that, while it associates the etiology of rheumatic fever definitely with streptococci, it declares in favor of no particular species or type strain of these microorganisms, since the allergic reactions may be excited by streptococci which differ widely both in biologic characters and in serologic relationships. That is to say that animals sensitized to one variety of streptococci, react not only to the homologous antigen, but to antigens derived from heterologous strains.

Zinsser and Yu[26] do not attempt an explanation of the mechanism of this bacterial allergy "further than to say that evidence seems to us to point toward the conclusion that the allergic state is a part of the immunologic mechanism, and in its first stages, before antibodies have been accumulated to any extent in the circulation, the phenomenon is mainly cellular, expressing itself in an extraordinary irritability of the cells to contact with the antigen. That with some bacteria, such as streptococci, they may later develop into a true immunity, or anergy, in other words a state in which contact with antigen does not produce reactions."

Dochez and Stevens[27] have described in animals which had received injections of streptococcus of erysipelas, the existence of two types or phases of hypersensitiveness, one to the toxin which can be neutralized by antiserum in vitro, the other, to some other bacterial product which they were unable to neutralize. The first sensitizing antigen was destroyed by heat and was therefore referred to as a toxin, while the second was thermostable. Birkhaug[28] found that animals sensitized with his nonmethemoglobin-forming streptococcus (Strain R. F. 24) showed likewise two phases of skin sensitivity to culture filtrates. The first appeared specific in that it was elicited only by filtrates of the homologous strain, and by those of Streptococcus cardioarthritidis. Homologous and crossed neutralization of the filtrates of these two strains was ef-

fected by their antisera, while heating of the filtrates at 98° C. for four hours completely eliminated the skin reaction in the earliest stages of this sensitization phase. During the second phase of sensitization neither antiserum nor heat had any effect in eliminating the skin reactions. During this stage also the animals reacted with positive skin tests to products of heterologous bacteria, Streptococcus viridans, Streptococcus hemolyticus of scarlet fever and erysipelas and pneumococci.

These observations suggest that the products of streptococci are complex substances, containing a multiplicity of protein substances each of which may act as antigen to produce its particular type of hypersensitiveness. In this particular they appear similar to horse serum or to egg albumen concerning which Von Dungern[29] has brought forth evidence to show that they contain a multiplicity of protein substances, each of which acts as antigen for the production of its own specific precipitins. The precipitins do not appear in the blood serum of the immunized animal in the same relative proportions in which the antigens occur in the horse serum, or in the egg albumen. Upon this observation then might hinge the explanation of the late appearance of the secondary phase of hypersensitiveness in animals treated with the complex antigens of streptococci.

Dochez and Stevens,[27] and Birkhaug's[28] observations that the late secondary phase of this hypersensitiveness is nonspecific, i.e., it appears as a hypersensitiveness to quite diverse serologic strains of streptococci, which also produce quite different toxic products, and appears also to pneumococcus, suggests that the bacterial bodies of such diverse microorganisms have in common some highly organized protein, which acts as a "protein specific" antigen rather than as a "species specific" antigen. We are familiar with such antigens from the work done with lens substance of the vertebrates, the immunologic specificity of which is not limited to a particular species, but occurs through a wide range of animal species as an "organ specific" antigen.

Swift's work in sensitizing animals with the nucleoproteins of streptococci, in which he finds that the antigenic properties of these cross the bars of immunologic type specificity in the streptococcus group and even invades the pneumococcus group, might be taken as evidence that in the nucleoproteins of bacteria (at least the gram positive cocci) we have such a "protein specific" antigen. The failure to find evidence of joint lesions, and of cardiac lesions in animals exhibiting extreme grades of this hypersensitiveness to nucleoproteins, as well as the failure to elicit focal symptoms referable to the joints and heart in patients with rheumatic disease, raises a serious doubt as to whether this type of hypersensitiveness has a significant part in the causation of the disease. The exhibition of the cross sensitization of this type between the streptococcus and the pneumococcus group, constitutes an argument for those supporting the view of nonspecific bacterial allergy as a dominant factor in the pathogenesis of rheumatic fever, that the disease might as readily be caused by pneumococci as by streptococci. Such reasoning, however, would do much to break down our whole conception of the specificity of bacterial disease, which is too well grounded to be shattered by an imperfectly understood laboratory observation.

THE AUTHOR'S HYPOTHESIS REGARDING THE FACTORS CONCERNED IN THE ETIOLOGY OF RHEUMATIC FEVER

The present state of our knowledge concerning the etiology and pathogenesis of rheumatic fever is too fragmentary to formulate any definite underlying principles except on the basis of hypothesis. There seems to be rather definite evidence that streptococci are the bacteria concerned in its causation. The evidence which my studies have brought forward suggests that a specific streptococcus is primarily responsible. The work of Swift[24] and his coworkers, of Zinsser and Yu,[26] of Irving-Jones[30] and others favors the view that a specific streptococcus is not necessarily concerned.

I am given the credit of favoring the type specific strain of streptococcus as the cause of rheumatic fever, acting through the specific toxic product alone. This but imperfectly presents my idea of the disease. It has been largely through the writings of others that my accredited position has been assigned to me. Swift is not convinced of a specific toxic element, and declares in favor of the nonspecific allergy to the various varieties of streptococci.

The studies of patients with rheumatic fever during the past two years have forced upon me the conclusion that there is an element of specificity to the Streptococcus cardioarthritidis in rheumatic fever. This element of specificity seems to be concerned with the "destructive" and "proliferative" lesions more so than the "exudative" ones. The proliferative lesion is the most important lesion in rheumatic fever, since it is the lesion most concerned in producing cardiac damage and since we have in salicylates a means of controlling the exudative lesion. The proliferative lesion, furthermore, is the lesion upon which the clinical and pathologic definition of the disease so largely depends.

It is desired to state clearly my present working hypothesis in regard to rheumatic fever, which is that the "destructive" and "proliferative" lesions are the result of a specific toxic product (endotoxin) derived from a particular group of streptococci. The destructive lesion is brought about by high concentrations of this toxin; the proliferative lesion, because of the stimulating effects of weaker concentrations. The exudative lesion appears when the patient begins to develop immunity to the specific toxic factor and is brought about by the establishment of a condition of hypersensitiveness which is a manifestation of the Arthus Phenomenon.[31]

From the work cited above it appears very likely that there are multiple factors concerned in this hypersensitiveness, some of which may be bacterial species specific, others specific only to certain highly specialized bacterial proteins. Because of the multiple antigens contained in streptococci, namely the specific endotoxin and the protein factors, one must presuppose the existence of separate and distinct antigen-antibody complexes. Of these the antigen-antibody complexes of the protein factors appear to be the most powerful irritating substances and would naturally be regarded as the dominant allergic factor, admitting the presence of other less potent allergic factors, e.g., the toxin-antitoxin complex.

This conception in no way disturbs the specificity of the destructive and proliferative lesions of rheumatic fever, but at the same time, it indicates

that the exudate lesions may be accounted for on the basis of bacterial "protein" specificity and not on the basis of bacterial group, or type specificity. It furthermore relegates the exudative lesions to a secondary, purely concomitant rôle in the disease and permits the presumption that any microorganism supplying the "protein specific" antigen to which the patient has developed a hypersensitiveness might play a part in determining the severity of the exudative lesions.

Fig. 1 presents a graphic representation of the interrelation of toxic, antitoxic, and allergic factors as applied in our hypothesis to the rheumatic diseases. It represents the allergic factor as increasing at the same rate as the antitoxic factor. The antitoxic factor is represented on a different base line from the toxic and allergic factors, because by this means its effect in counteracting the amount of the free toxic factor could be represented best. The

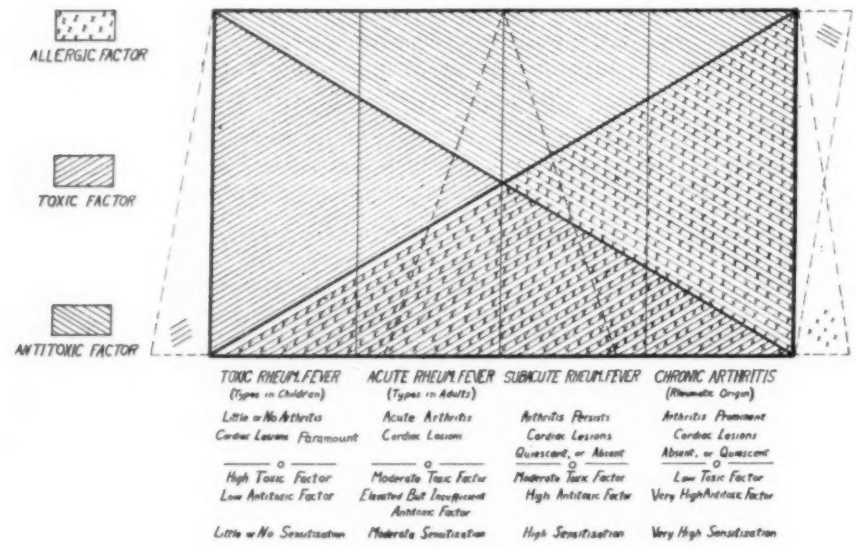

Fig. 1.—A graphic representation of the interrelation of the factors entering into the pathogenesis of rheumatic disease according to the hypothesis presented.

figure is divided into four vertical zones to illustrate in a general way the conditions of the hypothesis as applying to four types of clinical cases. The conditions applying to the individual case may be represented as those projecting on a vertical line through the figure. With these three factors operating as indicated in the hypotheses, it can be readily seen that a case as represented by a vertical line through the extreme left of the figure presents entirely different underlying conditions from one represented by a line through the extreme right, so that one might expect a difference in clinical manifestations as unlike as those of acute rheumatic carditis in children in the first instance and a low grade chronic atrophic arthritis in the latter. Yet each of these might arise primarily because of contact with the same streptococcus. The nonspecificity of the sensitization factor as to serologic type of streptococcus, however, does not limit the manifestations of chronic arthritis to a single specific streptococcus, whereas the manifestations of the acute rheu-

matic carditis are limited to a single specific streptococcus because of the biologic specificity of the toxic factor.

The problem in the acute rheumatic carditis thus appears to be that of adequate neutralization of the toxic factor without transferring to the patient hypersensitization to the protein of the streptococcus. The problem as represented for the chronic arthritis is not that of increasing immune bodies which are already present in quantity sufficient to neutralize the small amount of the toxic factor as it is formed, but rather the elimination of the condition of hypersensitiveness which the patient has probably acquired by virtue of the building up of the existing immune bodies. In the former instance the indication is for antiserum; in the latter, for minute "desensitizing" doses of the bacterial protein as available in saline extracts of the bacteria.

Cinically we see acute rheumatic fever without severe arthritic involvements. This is common in the first attacks in early childhood; nevertheless, children are especially susceptible to the destructive and proliferative lesions within the heart. The attack of acute rheumatic fever develops with an acute upper respiratory infection, and the patient is sick and toxic for from three to five days in advance of the onset of the arthritic involvements. During this period cardiac damage begins before the joint lesions appear. This early phase represents, or the disease in children who develop no joint symptoms, represents the purely toxic stage of the disease. This is the condition which I hold to be due to a specific toxic factor, which may be neutralized by its specific antiserum. According to this conception, the appearance of acute arthritis is a favorable sign, not only for pointing out the clinical diagnosis, but it is to be regarded also as indicative that the patient is beginning to build up his immune bodies.

In patients treated with the antiserum of Streptococcus cardioarthritidis, the prompt clearing up of the appearances of a severe clinical toxemia is an outstanding feature. A second feature of great importance is what I have termed the "focal" reaction following antiserum. This arises regularly when large doses of the antiserum are administered and manifests itself by the prompt appearance of new joints involved in an acute arthritis. For some reason this arthritis almost always affects the small joints of the hands. With this reaction large joints primarily involved usually clear up promptly. Untreated, the joints involved with the focal reaction subside in two or three days. Salicylate administration affects them favorably. Salicylate administration before administering antiserum, tends to prevent these focal reactions. During the focal reaction the patient's temperature remains elevated and fails to show the remissions which are common in the untreated case. The leucocyte count is elevated, at times very greatly elevated. The skin loses its characteristic pallor, and sweating is not troublesome. The joints involved in the reaction are red and swollen, but not particularly painful, and they are less tender than one would expect.

In our hypothesis of rheumatic fever this focal reaction represents the Arthus Phenomenon. The antiserum has supplied antitoxin, as well as antibodies of the protein factors of the streptococcus. The former detoxifies the patient and protects against further damage to the cardiac muscle. The lat-

ter by uniting with the protein factor of the infecting streptococci provides the antigen-antibody complex of the dominant allergic factor in such amount as to aggravate the exudative lesions of the joints.

In a practical way, this reaction (an exudative phenomenon) is ameliorated by giving the antiserum in small, divided doses, thus bringing about fractional combinations of antibody and antigen with the idea not only of reducing quantitatively the severity of the reaction, but also with the hope that such fractional production of the antigen-antibody complex will serve in partially desensitizing the patient against it. Or better still is the combining of the broken dose method with the use of moderate doses of salicylate by mouth.

Another observation of practical importance is concerned with the reactions following the injection of the vaccine, or of the soluble products of Streptococcus cardioarthritidis. The soluble product in use is prepared by suspending the killed microorganisms, washed free of the liquid media in which they are grown, in normal saline in concentration of 100 million microorganisms per 1 c.c. After seven days in the refrigerator, the bacteria are removed by filtration. The soluble products are obtained in the saline menstruum. This is diluted with normal saline for use clinically. At first it was employed in dilutions of 1:10 and 1:100; later, as these dilutions proved too potent, weaker dilutions were prepared. At present dilutions of 1:100 millions, 1:10 millions and 1:1 million are used. It has required more than eighteen months of study to reach the present minute doses of this product. It is noteworthy that the starting dose in a course of treatment is now $\frac{1}{20}$ c.c. of the 1:100 million dilution. Some conception of the minuteness of this dose is gained when one considers that this represents $\frac{1}{20}$ of the soluble extract from one streptococcus. Based on nitrogen determinations on the undiluted extract, the initial dosage contains one-twenty billionth of a milligram of protein. Yet with such amounts definite focal reactions in the joints, and systemic reactions occur in acute rheumatic fever, as well as in chronic arthritis of atrophic and hypertrophic forms. We believe that, even if this means nothing by way of specific bacterial sensitization, it does represent one of the most outstanding examples of hypersusceptibility to a bacterial product thus far recorded in medical literature. In our scaling downward of the dosage, it was noted that the injections were followed by definite general and focal reactions in patients with chronic arthritis and that such patients lent themselves better to the study of these extracts than did patients with acute rheumatic fever in whom harm was likely to result from improperly adjusted dosage. With the larger doses these reactions came on slowly, requiring from three to five days to reach their height. As the latter small doses were approached it was noted that they came on more promptly, and at present definite reactions are noted with doses as small as $\frac{1}{20}$ c.c. of the 1:100 million dilution, which come on as early as from three to eight hours after the injection. This tardy appearance of the reaction with the larger doses, and its prompt appearance with the small doses is an observation which cannot be definitely explained at present.

On the basis of the hypothesis offered above it can be accounted for in the following manner: The individuals showing these responses have some grade of immunity. The introduction of antigen into them permits the union of the antibody which they possess with this antigen, thus forming the antigen-antibody complex to which they exhibit a hypersensitiveness. The symptoms of the reaction are in this conception a manifestation of the Arthus Phenomenon. But for the exhibition of the Arthus Phenomenon, this union alone does not furnish the proper conditions. These two factors must be combined in the proper relative proportions. An excess either of antibody or of antigen in the complex prevents the appearance of this reaction. The larger doses of antigen introduced into patients furnish an excess of the antigen factor, so that the appearance of the reaction is delayed until this excess is destroyed or eliminated. With the very small doses used recently, proper conditions are at once established and this union of antigen and patient's antibody occurs in the proper relative proportions for the prompt manifestations of this reaction. Bringing these considerations back to the patient, these prompt general reactions following the small doses of antigen appear to be exudative phenomena, in that they are ameliorated by the oral administration of salicylates. This further suggests that they arise from the dominant allergic factor, the antigen-antibody complex of the protein antigens of bacteria, rather than from the toxin-antitoxin complex. As such the protein antigens of various bacteria would give rise to them to a greater or less degree. Heating of the soluble extracts of the bacteria would not eliminate the reaction. Both of these conditions apply as will appear later under the discussion of chronic arthritis.

This working hypothesis might be pursued further to raise such questions as: Are these small doses of antigen sufficient to build up a permanent immunity? The evidence at present indicates that they may not be. Do they merely desensitize the patient? If they do, is it possible after desensitizing with small doses of antigen, to practice the giving a few large doses of vaccine to build permanent immunity, or whether the permanent immunity, if at all possible, may not have to be established by the use of the antigen-antibody mixtures prepared in vitro? But these considerations while very important in the solution of the problem, belong to the realm of the rapidly advancing science of immunology.

The manner of preparing the soluble antigen, as well as the extremely small dosage tolerated by rheumatic patients and its apparent harmlessness to normal individuals suggest that it may be only the desensitizing agent. On the other hand its ability to precipitate relapses which exhibit both the exudative and the proliferative types of lesions in patients convalescing from rheumatic fever, suggests that it contains all of the substances entering into the production of the disease. According to our hypothesis it might be presumed to have both the endotoxin and the various protein sensitizing antigens. Work which has just been started, in which the antigen heated at 100° C. for two hours is employed in patients with chronic arthritis, shows that definite desensitizing phenomena are obtained. This indicates that thermostable protein antigens are present, but our studies have not progressed far enough to

give definite indications that a thermolabile antigen has been eliminated from the product by the heating process.

Our earlier use of vaccines in dosage equivalent to many times those represented by the antigen dilutions now used may have been more effective in inducing active immunity. Their use, however, was decidedly unsafe. It is not improbable that they may be employed effectively and with safety in patients desensitized by means of heated soluble antigens. The principles underlying the active immunization of patients against pathogenic streptococci, to some of the products of which they exhibit a hypersensitization, constitutes one of the greatest needs of present medical practice. Upon the development of these principles depend any advances which will be made in the specific prophylaxis of rheumatic fever with its appalling toll of cardiac disease.

In closing I must mention chronic arthritis, because our experience with the vaccines and antigens of Streptococcus cardioarthritidis has shown that there is a relationship between rheumatic fever and that group of inadequately classified arthritides commonly spoken of as atrophic, hypertrophic, rheumatoid and deforming arthritis. This relationship may, however, be more apparent than real. These diseases appear to be on a purely allergic basis, an allergy which may be that due largely to "protein specific" antigens common to streptococci, pneumococci and perhaps other microorganisms and in which species specific antigens may play a minor rôle. The fundamental problem in their treatment appears to be that of adequate desensitization. Since Streptococcus cardioarthritidis contains this nonspecific allergizing antigen its products become effective in this desensitizing process.

Saline extracts of Streptococcus cardioarthritidis have been used largely and effectively in the treatment of these cases. As mentioned above they still retain desensitizing properties after prolonged heating at 100° C., and it is probable that the heated product will eventually prove more practical than the unheated, which possibly may contain a subtle endotoxin. Our experience, however, thus far has been largely with the unheated product, and upon experience with it, the following statements are based. Improvement attends its use in this group of patients. This appears more striking and permanent where the closed foci harboring streptococci have been eliminated. Employed in excessive dosage, no improvement or actual activation of the disease results. Focal reactions in the joints, such as pain, stiffness and swelling are constant following injections. Occasionally redness of the skin over the acutely swollen joint follows the injection. Gastrointestinal symptoms, anorexia, nausea, vomiting, hyperperistalsis, also nervous and emotional symptoms, tremors, insomnia, irritability, melancholia, uncontrollable crying, etc., are frequently observed.

Soluble extract has been prepared from a number of strains of Streptococcus viridans, all of which had been isolated from blood cultures in subacute bacterial endocarditis. These have been prepared in the same manner as the soluble antigen of Streptococcus cardioarthritidis and used in similar dilutions in patients with chronic arthritis, i.e., 1:100 million and 1:10 million dilutions. With these extracts reactions similar to those obtained with the soluble antigen of Streptococcus cardioarthritidis are being obtained in these

patients. This observation further strengthens the hypothesis that streptococci contain a common protein factor to which patients with arthritis are hypersensitive, and suggests that this hypersensitization is perhaps the basic underlying condition which maintains the chronicity of the arthritis. The lack of streptococcus type specificity in this hypersensitiveness is in accord with Swift's experimental work on bacterial allergy.

From the standpoint of the treatment of the patient with chronic arthritis, these observations have a particular significance, because they suggest that we need not of necessity obtain the particular strain harbored by the patient for the preparation of a vaccine or a soluble extract to be used in treatment. Practically any streptococcus contains the antigen to which the patient is hypersensitive and therefore stock preparations rather than autogenous ones may be used effectively. This simplifies very much this form of treatment of chronic arthritis in two particulars. First, it avoids the isolation of streptococci and preparation of autogenous streptococcal products; and, second, it enables the use of a stock product which can be standardized more exactly as to dosage requirements. This latter consideration is one of extreme importance because there appears to be a rather narrow margin of safety between the effective therapeutic dosage and harmful overdosage.

For the adjustment of these dosage requirements to the patient's needs it has been found more practical to employ saline extracts of streptococci rather than vaccines. While our practice of very minute dosage of the bacterial extracts is in line with the developments of Warren Crowe and of Burbank in the treatment of chronic arthritis with small doses of vaccine, it utilizes dosage which is too small to be obtainable with vaccines, e.g., the $\frac{1}{20}$ of the soluble extract of a single streptococcus. The use of the soluble extracts of streptococci therefore cannot be compared with that of minute doses of vaccine. It is more strictly analogous to the pollen extracts used in hay fever, or to tuberculin. Since precautions are taken to prevent autolysis during the saline extraction of the bacteria, these extracts are not of the nature of the various bacterial autolysates employed in therapy. We believe, therefore, that their preparation and use in minute dosage represents a refinement in immunotherapy and that clinical results are obtainable which would be impossible from the use of either bacterial vaccines, or autolysates in dosages commonly employed.

Th employment of this form of therapy does not relieve the physician of the arduous task of identifying and eliminating focal infections. One could not expect "desensitization" treatment to be effective in the presence of a closed "sensitizing" focus of infection, any more so than could he expect the desensitization treatment of hay fever from sensitization to ragweed pollen to be effective in a patient exposed to this pollen at the height of its season.

SUMMARY

The rôle of streptococci in rheumatic disease is discussed, and an hypothesis embracing a dual nature of its pathogenesis is offered, as follows:

The specific toxin of a streptococcus is suggested as operative in the production of the "destructive" and "proliferative" types of lesions in rheu-

matic fever, while the patient's hypersensitization to an allergen associated with the protein of streptococci is presented as concerned in producing the "exudative" lesions. The specificity of this sensitizing substance, or allergen is not dependent upon the type of streptococcus supplying it.

Chronic arthritis is presented as an allergic disease, the hypersensitive state of the patient being due to this allergen contained in streptococci without regard to a particular type.

The specific treatment of rheumatic fever embraces both the neutralization of the toxemia, and adequate desensitization.

The treatment of chronic arthritis is concerned largely with the elimination of foci harboring streptococci, and with adequate desensitization.

Stock extracts of streptococci may be employed in this desensitization, and because their dosage requirements may be the better determined through extensive clinical application they are preferable to autogenous preparations.

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PRIMARY SARCOMA OF THE SPLEEN (RETICULUM CELLED)*

Report of a Case

By Tasker Howard, M.D., Brooklyn, N. Y.

PRIMARY sarcoma of the spleen is a rare disease. Krumbhaar[1] encountered four cases in 6,500 autopsies. Smith and Rusk[2] found in the literature 104 cases of primary malignant disease of the spleen, all of which they considered sarcoma. Their paper includes an abstract of most of these cases, and they add a report of two additional cases to this list. Since that time cases of primary sarcoma of the spleen have been reported by Ladreyt[3] (one), de Brun[4] (one), Beasley[5] (one), Birman[6] (one), Köhler[7] (one), Adam[8] (one), Eichenbusch[9] (one), and Krumbhaar[1] (four). The record of another example of this disease follows:

Case Report.—Miss M. H., seventy-three years of age, single, a retired school teacher, born in the United States, was admitted to the Long Island College Hospital the first time November 8, 1926, complaining of fever, diarrhea, and weakness.

The *family history* was irrelevant.

The *previous history* included an attack of pneumonia as a child, and a tendency to an occasional digestive upset which apparently most often took the form of diarrhea. She had been told twenty-five years previously by Dr. Jacob Fuhs that she had an achlorhydria. Five years before admission she had a diarrhea which lasted the better part of the winter and was finally controlled after treatment in a hospital in California. For several years she had been known to have a blood pressure which ran over 200 mm. systolic most of the time.

The onset of the *present illness* was dated by the patient as about six weeks before admission, when she was rather suddenly taken with diarrhea and fever while travelling in France. At about the same time she noticed some abdominal soreness and a mass in her abdomen. She was in bed several weeks before she was able to return home and kept in her bed on the steamer, coming to the hospital within two days of landing. Meantime she had grown very weak and had noticed some swelling of the legs. The diarrhea had largely subsided, though the bowels still tended to be a little loose. She had never noted soreness of the tongue or mouth, or numbness of the hands or feet. She stated that she had been of a sallow color, possibly a little yellow for a long time.

On admission her temperature was 100.4, pulse 100, and respiration 26. She was rather poorly nourished, and sallow in color, showing considerable pigmentation of the hands and face. There was nothing significant in the examination of her head and neck, except the absence of any evidence of glossitis or stomatitis and the absence of adenopathy.

The chest was of the normal type and the lungs quite negative to examination. The heart seemed enlarged and presented a "squealing" systolic murmur, which was audible all over the chest. The arteries were definitely thickened, and the blood pressure 180 mm. systolic, 110 diastolic.

In the abdomen a splenic tumor dominated the picture. It extended 4 cm. to the right of the midline and down to the iliac crest. The notch could be readily felt. The organ was smooth, hard and not tender. The liver edge was also palpable below the level of the umbilicus.

*From the Medical Service of the Long Island College Hospital.
Received for publication, December 8, 1928.

The extremities showed no edema. The knee jerks and ankle jerks were absent.

The Laboratory Data:

Urine: 1015, albumin faint trace, few hyaline casts and leucocytes. No bile present. Urobilin was not looked for.

Gastric analysis: Achlorhydria, total acidity 12, otherwise not noteworthy.

Blood Wassermann: Negative.

Blood:

Date	Hb.	W.B.C.	R.B.C.	Polys.	Large monos.	Lymphs.	Eosins.
11/ 8	50	3,200	2,550,000	62	26	12	0
11/12	48	2,800	2,496,000	54	28	16	0
11/18	49	2,200	2,600,000	61	24	14	0
11/23	53	3,200	2,700,000	68	13	17	2

The index in these counts is just about 1. No nucleated reds were seen. There were some irregularities in the shape and size of the red cells, and some polychromasia was noted. The high large mononuclear count is noteworthy. The platelet count was 288,000.

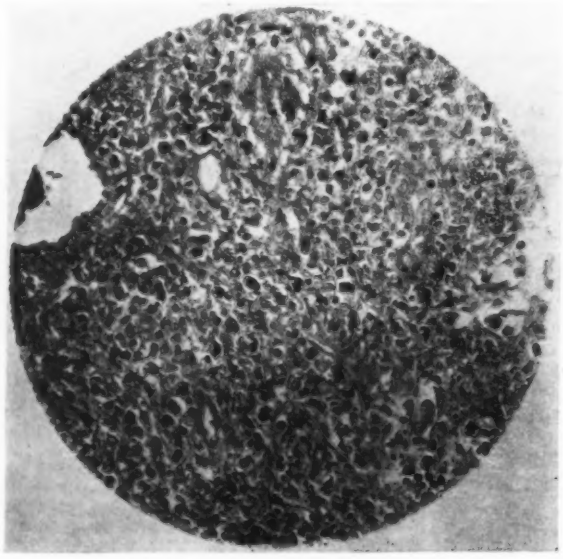

Fig. 1.—Reticulum celled sarcoma of the spleen. The microphotographs were taken by Mr. J. V. Dunn.

The fragility was found to be slightly less than that of a normal control. Hemolysis started at 0.36 per cent and was complete at 0.27 per cent, the figures for the control being 0.40 per cent and 0.32 per cent. Fifty reticulated cells per thousand were found by vital staining.

The icterus index was 7 on November eighth and on November sixteenth it was 8.

The van den Bergh reaction showed a delayed direct response and an immediate indirect response on two occasions.

Course of the Disease.—In the course of three weeks, with rest in bed, sunshine, and fresh air, hydrochloric acid and arsenic and iron, she made a great advance symptomatically, although it will be noted that there was but little change in the blood count. She was allowed to go home, where she continued to gain, her hemoglobin reaching 76 per cent by the end of January. Part of this period she was supposed to be upon the Minot and Murphy liver diet, although not until she had made the greater part of the gain ultimately attained. It was difficult for her to follow the prescribed diet and she never entirely succeeded. After January she began to lose ground, becoming paler and weaker, with a

gradual diminution in the blood count. By the middle of June she was obliged to return to the hospital, her hemoglobin having fallen to 46 per cent, her red cells being 3,072,000 and her leucocytes 3,200. At this time she developed an upper respiratory infection, to which she was quite prone. She exhibited an irregular fever and a tendency to excessive sweats. She gradually failed and died July 16, 1927. There was no particular change in laboratory findings or physical signs at the time of her second admission. During this period she was under the care and observation of Dr. George H. Roberts.

Autopsy.—The spleen is enormously enlarged, weighing 1800 gm. The enlargement is regular so that the normal configuration of the organ is retained. At the hilus and along the lienal artery are enlarged and congested lymph nodes. A large part of the surface of the spleen is grey-white in color suggesting extensive infarction. The remainder of the surface is of a dark brick-red color. The consistency is slightly firmer than normal. Over the outer surface are found some recent adhesions. On section the normal splenic structure is entirely lacking, much of the tissue being replaced by an irregular caseous looking mass. Just below

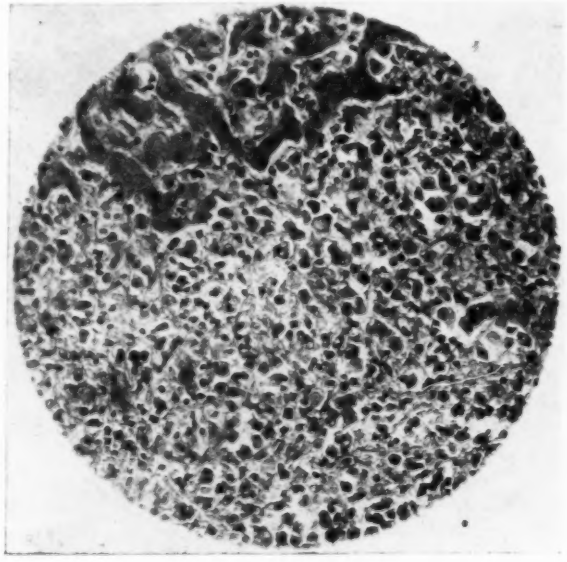

Fig. 2.—Secondary invasion of the liver.

the cortex this tissue is most abundant, accounting for the surface picture above noted. Between these areas the tissue is dark red in color and much of it is hemorrhagic.

The liver is large and pale.

Numerous large retroperitoneal lymph nodes are encountered which on section are uniform in structure and opaque.

Microscopic.—Lymph Nodes: All of the enlarged lymph nodes show the same structure. The normal architecture is entirely replaced by a new growth. The cell type is that of a reticulum cell with round or ovoid vesicular nucleus and fairly abundant cytoplasm. Giant forms are occasionally encountered and numerous mitotic figures are seen. Among these cells are scattered varing numbers of lymphocytes and a delicate reticulum is present throughout.

Spleen: Much of the tissue is necrotic and a good deal of congestion is present throughout. In certain areas the cells are fairly well preserved and the structure is seen to be identical with that of the lymph nodes.

Liver: The new growth has invaded the liver and much of the parenchyma has been replaced. The appearance here is much the same as seen in the lymph nodes. The distribution of the tumor throughout the liver does not seem to be related to the anatomical structure

of the organ, central and peripheral portions of the lobules being equally involved. The remaining liver cords show marked cloudy swelling.

Diagnosis.—Reticulum celled sarcoma.

There are some points about the case as it presented itself clinically that are worth noting. In many ways pernicious anemia was strongly suggested, as in (1) the grave anemia with the high index, (2) leucopenia, (3) achlorhydria, (4) high icterus index and indirect van den Bergh, (5) the apparent remission, which to the patient's mind was practically complete, and the relapse, and (6) the suggestion of cord involvement in the absent reflexes. However, the spleen was larger than occurs in this disease, and several other minor points, such as the absence of glossitis, megalocytosis, and thrombocytopenia, made this diagnosis unlikely. Chronic hemolytic jaundice could be ruled out

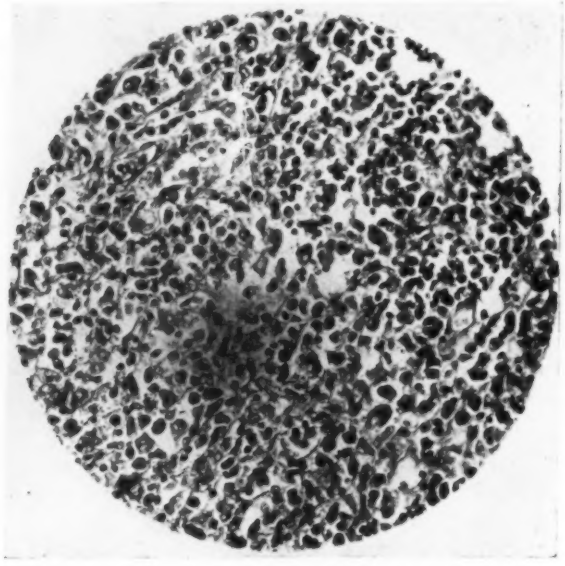

Fig. 3.—The same tumor involving a lymph node.

by the normal fragility of the erythrocytes. In Banti's disease the anemia is more characteristically of the secondary type, and evidence of active hemolysis is seldom as frank. The clinical diagnosis was hemolytic anemia with splenomegaly of undetermined character. Splenectomy was considered, but in view of the age of the patient, the condition of her vascular system and blood, the hepatic enlargement, and the severity of the operation, it was not performed. Splenectomy for primary sarcoma of the spleen has been performed successfully in 21 of 39 cases collected from the literature of Smith and Rusk.[2] Of the 18 who died, 7 succumbed to the operation and 11 to recurrences.

The type of anemia in these cases is of some interest. Blood counts are available in 25 of the cases cited. There was some degree of anemia in all but one and a high degree in a number of them. The color index was reduced in about half of them and was not reduced in half. The increase in large mononuclear cells noted in this case was not reported in any of the others.

Fragility was tested in but very few cases, and as in this case it proved normal when tested.

No record was found of any studies of the bilirubin by means of the icterus index or the van den Bergh test. The observations in our case seem to indicate a pathologic degree of hemolysis.

SUMMARY

To the 115 cases of primary sarcoma of the spleen found in the literature is added the report of an additional case which clinically strongly resembled pernicious anemia.

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THE EFFECT OF MERCURY AND SODIUM OXALATE ON BLOOD CALCIUM*

By F. D. McCrea, Ph.D., Augusta, Ga.

THIS series of experiments, the first of which is here reported, was undertaken to ascertain the effects of certain drugs upon the level of calcium in the blood.

Various investigators have reported that mercury changes the calcium metabolism of the body. Chiari[1] found that the calcium was diminished in the vascular elements, cells and nerves of the intestinal wall, and Koeniger[2] cited by Biberfeld, stated that the calcium content of the blood almost never increased, as a result of the administration of mercury, in acute experiments. Klemperer[3] and Schmiedeberg,[4] however, report that there is no modification in the calcium of the blood as a result of the administration of mercury.

That the metabolism of calcium in the body is not influenced by mercury, in spite of the presence of crystals of calcium phosphate in the kidney, is stated by Heilborn,[5] Binet,[6] Salkowski, Kaufmann, and Prevost and Eternod.[7] Several authors, Heilborn,[5] Sabbatani,[8] Jablonowski,[9] Prevost and Fürbringer,[10] and Senger[10] report that mercury has a decalcifying action upon the osseous tissues, which become fragile and poor in lime.

The determinations were made upon animals in which the toxic effects of mercury were being investigated. This was done at the suggestion of Dr. Salant (personal communication), who thought there might be some causal

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relationship between the toxicity of mercury and the calcium content of the blood, since the action of mercury upon the heart in many of his experiments was suggestive of a disturbance of the normal calcium-potassium ratio.

EXPERIMENTAL METHOD

Healthy cats were used under urethane anesthesia, with the exception of one experiment, in which ether was used. The animals were tracheotomized, both femoral veins, and the right carotid artery cannulated, the former for the administration of the drug, the latter for the registration of blood pressure, respiration also being recorded.

Mercury succinate was injected into the femoral vein at the rate of one milligram of mercury per kilogram of body weight at intervals of fifteen minutes, until sufficient mercury had been administered to cause death. Blood was taken from the carotid artery for the control determination, the final sample being obtained from the heart immediately after death. In those experiments in which only normal blood was analyzed, samples were drawn directly from the heart. Blood calcium was determined by the method of Roe and Kahn,[11] all determinations being made in triplicate.

The blood calcium was studied in animals on different diets, and determinations were made before and after the administration of mercury, in the majority of the experiments.

RESULTS

It will be seen by examination of Table I that animals fed upon a diet consisting entirely of salmon had a higher control level of blood calcium than did those fed solely upon beef, or those which had been starved for seven or eight days. The lowest initial calcium reading was obtained from an animal brought in off the street and fed chopped beef for five days. The calcium

TABLE I

EFFECT OF MERCURY ON BLOOD CALCIUM

EXPERIMENT NUMBER	MG. CALCIUM PER 100 C.C. BLOOD. CONTROL	LETHAL DOSE MERCURY IN MG. PER KG.	MG. CALCIUM PER 100 C.C. AFTER HG.	DIET
690	9.80			Bread and Salmon
691	8.71			Bread and Salmon
692	8.59			Bread and Salmon
693	9.27			Bread and Salmon
714	8.62			Bread and Salmon
715	9.68			Bread and Salmon
694	11.70	12	11.38	Salmon
699	13.78	14	9.89	Salmon
700	11.06	18	8.87	Salmon
702	11.26	16	9.29	Salmon
706	9.90	5	10.10	Bread and Salmon
707	8.08	11	7.06	Bread and Salmon
716	9.78	24	7.17	Starved 7 days
717	8.17	10	8.45	Starved 8 days
719	9.51	14	6.97	Beef 3 days
720	9.51	11	7.26	Beef 4 days
721	9.52	7	7.74	Beef 2 days
722	8.42	6	7.27	Beef 5 days
723	7.85	13	4.94	Beef 5 days
724	8.98	10	7.18	Beef 8 days

level of the remainder of the beef fed cats was little different from that of the animals which had been starved. However, the duration of the beef feeding period was probably too short to warrant definite conclusions being drawn.

The average control level of calcium in the entire series of animals was 9.69 mg., whereas that of 5 salmon fed cats was 12.20 mg. per 100 c.c. of blood. The average of the beef fed and starved animals was the lowest of the series, namely, 8.97 mg., and those fed a diet consisting largely of bread, supplemented by small quantities of salmon averaged 9.11 mg. of calcium per 100 c.c. of blood.

Mercury in amounts ranging from 5 to 25 mg. per kilogram of body weight, produced a rather marked diminution of blood calcium in 13 of the 16 experiments. Animals 706 and 717 show an apparent increase, which, however, does not greatly exceed the limit of experimental error of the method, and number 694 shows a decrease of about the same magnitude.

Sarvonat and Roubier[12] report 5 experiments on guinea pigs, two being used for controls, in which the calcium of the skeleton and soft parts of the body were determined. Three were injected subcutaneously with sodium oxalate until death occurred, the average amount required being 0.195 gm. for animals of average weight. Skeletal calcium was decreased from 0.496 to 0.378 gm., or approximately 24 per cent, and the soft parts from 0.179 to 0.101 gm., or 42.5 per cent, per gram of ash.

In the determination of the mineral metabolism of a rabbit receiving 4 c.c. of 3 per cent sodium oxalate for three days, Luithlen[13] found an increased elimination of potassium and calcium, the former being greatest, little change in magnesium, and a retention of sodium.

To determine whether depletion of the blood calcium before the administration of mercury would have a different effect on the action of mercury, some animals were injected subcutaneously with sodium oxalate at the rate of 25 milligrams per kilogram of body weight, for periods varying from five to nine days. The results of these experiments appear in Table II. In those animals injected for the shorter period, five days, the initial calcium level, before oxalate, was near the level of the entire control group. Three animals, Nos. 709, 710, and 715, which were injected for nine days, and one, No. 714, injected for eight days had a definitely low calcium content. The administration of mercury to these animals was followed in five of the six animals by the further reduction of the blood calcium, while one, No. 701, was unaffected.

TABLE II

SODIUM OXALATE SUBCUTANEOUSLY FOLLOWED BY MERCURY

EXPERIMENT NUMBER	MG. CALCIUM PER 100 C.C. BLOOD CONTROL	LETHAL DOSE HG. IN MG. PER KG.	MG. CALCIUM PER 100 C.C. AFTER HG.	NUMBER DAYS INJECTED WITH $Na_2C_2O_4$
701	9.90	5	10.10	5
704	8.08	11	7.06	5
709	4.50	5	Too dilute to read	9
710	4.33	5	Too dilute to read	9
714	6.30	9	4.90	8
715	6.27	11	4.62	9

The question of variation of the calcium content of the blood as related to diet cannot be discussed, since no analyses of food intake and excreta were made. The average calcium content of the foods used, determined as calcium oxide, according to the tables of Sherman,[14] is as follows: Baker's bread 0.03 per cent, salmon 0.011 per cent, lean brisket 0.011 per cent, based on the edible portion. Total ash, from tables by Atwater and Bryant,[15] baker's bread 0.8 per cent, canned salmon 2.0 per cent, lean brisket 1.0 per cent. The figure of calcium content of salmon (Sherman) does not take into consideration the bone present in canned salmon, which would necessarily increase the percentage of calcium.

Possibly the fact that the cats fed exclusively on canned salmon shows a higher control level of calcium than the remainder may be in part explained by the statement that the coefficient of digestibility of salmon is about 12 per cent higher than that of bread (Sherman), and that when the diet was composed primarily of bread, peristalsis was probably greater. More calcium may have been lost in the excreta, and possibly less absorbed as a result of increased peristalsis, than in the case of the salmon fed animals.

SUMMARY

The administration of mercury succinate in acute experiments effects a considerable diminution of the calcium of the blood.

Blood calcium is diminished by the administration of sodium oxalate. Mercuric succinate administered to animals with low blood calcium results in a further depletion of serum calcium.

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STUDIES IN THE ALIMENTARY TRACT OF MAN*

II. Stabilization of Gastric Reaction Patterns

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INTRODUCTION

In A previous article we set forth our conviction that the fundamental necessity for a study of motility in the human alimentary tract is the employment of stomachs (or intestines) of subjects carefully trained in roentgenology so that subconscious influences which modify the alimentary responses may be reduced to a minimum. In the present paper we propose to examine this thesis further as a preliminary to the development of a technic of diagnostic meals. Actually for the moment, this resolves itself into a consideration of the physical condition of the stomach.

In clinical writings the terms gastric tone and gastric peristalsis appear to indicate a distinction between these two forms of gastric activity, and, indeed, one form may be so obtrusive that it eclipses the other. Both however are evidences of smooth muscle irritability. In a roentgenologic investigation they express themselves in area and shape of shadow and in the character of the gastric movements.

THE EMPTY STOMACH

On two different occasions (Sophomores, Nos. 314 and 104) we were fortunate enough to see on the fluoroscope the actual size of the empty stomach of the students before the barium meal was given. The gastric tube seemed to be reduced both laterally and anteroposteriorly, to the width and thickness of two fingers; it appeared about five to six inches in vertical height. We handed the prepared barium meal to the student, and asked him to hold it for a few minutes. Meanwhile we examined carefully the delicate stomach shadow on the fluoroscopic screen. We observed that the stomach shadow widened out and lengthened during this period as though it were becoming filled with gastric juice. This change in outline was completed within thirty seconds; we were unable to obtain measurements. It may be that the very delicate outline is responsible for our failure to observe peristalsis or indeed any movement. But it would be natural at least to expect, that, in a condition of emptiness, gastric movements would be less vigorous than when contents are present.

Since the above paragraph was penned we have had the opportunity of examining the same phenomena on several further occasions, always with the same result.

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Studies I-IV of this series form parts of a thesis for which the degree of A.M., was conferred by Western Reserve University upon Wilhelmine Kuenzel, June, 1928.
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TYPES OF FILLING

The precise method of entry of barium into the stomach varies and indeed the same stomach may show different types of filling at successive examinations. Nevertheless the number of methods of filling was greater when we first commenced our study than it is today and it is quite possible that increasing control over our subjects, as the tradition of routine examination becomes established, may result in a reduction to order of what is at present a rather chaotic medley of observations.

The development of the barium shadow may take place from above downward or from below upward. Of the first type there are four methods. In the first the barium spreads itself instantly throughout the cardiac sac. It then either passes immediately in a continuous mass through the gastric tube to the pylorus or it sifts gradually through gastric fluid, rapidly secreted into the gastric tube, to settle more slowly in the pyloric vestibule. Secondly it may be so held up in the cardiac sac as to give the appearance of an inverted barium cone from which a pencil-like stream trickles through the gastric tube to the pyloric vestibule. Thirdly it may outline the greater curvature only by a stream which however gradually broadens until, in some examinations, the lesser curvature is also outlined. In these three methods the essential principle is a filling of the cardiac sac with immediate or delayed passage to the pylorus, the time taken depending upon promptitude of relaxation of gastric musculature or speed of secretion of gastric juice. In the fourth and rarest method there is no spreading out in the cardiac sac but the barium passes down the lesser curvature, its shadow gradually widening (probably through gastric juice) until the greater curvature is also outlined.

There is a totally different method of filling in which it is difficult to follow the passage of barium from the cardia through the gastric tube unless, as sometimes, happens, it defines the lesser curvature in its passage. The barium first collects in recognizable amount at the lowest part of the pyloric vestibule which then fills from below until there is a heavy shadow of barium up into the gastric tube. This type of filling occurs more frequently in the elongated, vertically placed stomach. We were at first inclined to associate it with Freshmen only but Sophomores exhibit it also. In Sophomores indeed we have observed quite frequently the faint unoccupied outlines of greater and lesser curvatures and a filling from above by the barium stream which passes gradually down the gastric tube, midway between the curvatures, to the pyloric vestibule. A faint barium shadow outlines the curvatures and shows that the greater is opening out laterally. Since the Sophomore stomach is more constant in its responses than that of the Freshman we are inclined to believe that this method of filling should be considered the most natural one.

CHARTS OF THE GASTRIC FEATURES

In Chart 1 are represented the positions of cardia, pylorus, and greater curvature in all students examined between January, 1925, and October, 1927. The records are all from buttermilk meals, 16 ounces to the meal, except for the Sophomores in October, 1927, who had 5-ounce meals and whose record is listed separately. The total Freshmen are 86; the Sophomores somewhat less

through academic casualties. For each group the mode is easily recognized, the median is encircled and the mean position is designated by the letter *M*. Crude averages in series of such a dispersal do not inspire much confidence and indeed experience assures us that a difference of one unit is probably negligible, though an interval of two or more units is almost certainly significant. From this chart there seems to be no change in position of cardia or pylorus connected with duration of digestion or status of student. On the other hand greater curvature, though showing no change associated with duration of digestion or size of meal, undergoes an elevation in site in the change from Freshman to Sophomore. Small as it is the change noted in the chart is of im-

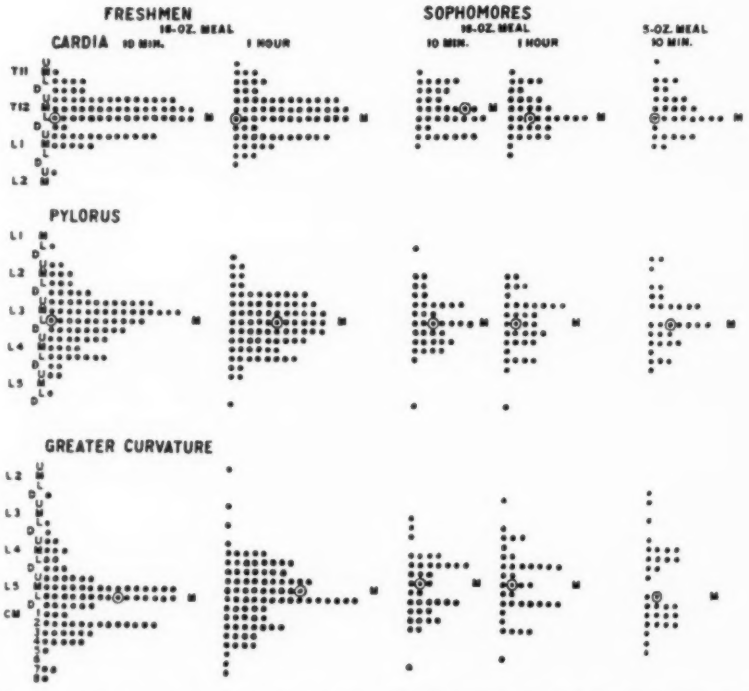

Chart 1.—Relation of stomach levels to student status, period of digestion and size of meal. Vertebrae indicated in thirds, upper, middle, and lower. Disc between fourth and fifth lumbar vertebrae also plotted. Median (encircled), mean (M) and mode all indicated.

portance. It was the first indication we had of the group response discussed in our last paper. It encouraged us to examine our individual records in detail and so discover the striking change which takes place in vertical dimension of the stomach with the progress of training.

With the data upon which the chart is made we have developed the summarizing Table I which, especially in the common averages, shows the rise of greater curvature from Freshman to Sophomore. Greater clarity could not be expected from distributions with so large a range. Hence the full expression and meaning of the differences can be determined only by examination of the individual records. This has been done and attention, in consequence, focused on shadow area but before the results of this work can be properly

presented we must have further assurance regarding the dependability of technical methods involved.

TABLE I

GROUP AVERAGE STOMACH POSITION

		CARDIA		PYLORUS		GREATER CURVATURE	
		10 MIN.	1 HR.	10 MIN.	1 HR.	10 MIN.	1 HR.
Group I							
Freshmen	Jan. 1925	LT12	MT12	LL3	LL3	LL5	UL5
Sophomores	Oct. 1925	MT12	LT12	ML3	UL3	UL5	dL4-5
Group II							
Freshmen	Oct. 1925	LT12	UL 1	LL3	dL3-4	LL5	LL5
Sophomores	Oct. 1926	MT12	LT12	LL3	LL3	UL5	UL5
Group III							
Freshmen	Oct. 1926	MT12	MT12	ML3	ML3	LL5	ML5
Sophomores	Oct. 1927	LT12		LL3		ML5	
Common Average Stomach Position							
All Freshmen		LT12	LT12	LL3	LL3	LL5	ML5
All Sophomores		MT12	LT12	LL3	LL3	UL5	UL5

PRACTICABILITY OF RECORDING CHANGE IN GASTRIC DIMENSIONS

To test the precision with which it is possible to determine gastric dimensions we have made two series of observations on width, height, and area of stomach shadow. The results are given in Table II.

TABLE II

CHECK RECORDS OF STOMACH DIMENSIONS

1. Width and Height

	NUMBER OF STUDENTS	TRIAL	AV. 10 MIN. WIDTH	AV. 10 MIN. HEIGHT
Group I (Freshmen)	29	1	81.4 mm.	251.2 mm.
		2	80.8	249.6
Group II (Sophomores)	31	1	51.4	210.7
		2	50.5	211.0

2. Area

	NUMBER OF STUDENTS	AVERAGE AREA 1ST TRIAL	AVERAGE AREA 2ND TRIAL
Group I (Sophomores Indoor Occupation)	8	11797 sq. mm.	11554 sq. mm.
Group II (Sophomores Outdoor Occupation)	9	12287	13031

Width of shadow was determined at the lower limit of the Magenblase, a site which by experience we know to be relatively stable. Vertical height is the maximum distance between highest and lowest points of gastric outline measured parallel to the vertebral axis. Area is obtained by planimeter as described in our former article.

In the series for width and height the first group consists of 29 Freshmen with 16-ounce buttermilk meals. The second group consists of 31 Sophomores with 5-ounce sweet milk meals. Due care was taken to confuse the memory

of the observer who made the determinations. The area determinations were made on Sophomore students with 5-ounce meals.

Although the results of this trial are more uniform for linear dimensions than for area, as might be expected, our findings in all measurements justify us in proceeding with the investigation.

DIMENSIONS OF SHADOW ON REPEATED EXAMINATION

The qualitative difference in gastric response of Freshmen and Sophomores has been our main theme both in this article and our former one. Table III shows the gastric reactions in terms of three measurable dimensions, width, height, and area.

TABLE III

RELATION OF STOMACH DIMENSIONS TO REPEATED EXAMINATIONS

1. Width				
	DATE	AV. FRESHMEN 10 MIN. WIDTH	DATE	AV. SOPHOMORES 10 MIN. WIDTH
Class of 1928	Jan. 1925	71.1 mm.	Oct. 1925	72.3 mm.
Class of 1929	Oct. 1925	80.7	Oct. 1926	83.1
Class of 1930	Oct. 1926	81.1		
	Av. Freshman width, 77.6 mm.		Av. Sophomore width, 77.7 mm.	
2. Height				
	DATE	AV. FRESHMEN 10 MIN. HEIGHT	DATE	AV. SOPHOMORES 10 MIN. HEIGHT
Class of 1928	Jan. 1925	252.1 mm.	Oct. 1925	230.6 mm.
Class of 1929	Oct. 1925	245.4	Oct. 1926	231.5
Class of 1930	Oct. 1926	250.4		
	Av. Freshman height, 249.3 mm.		Av. Sophomore height, 231.0 mm.	
3. Area				
	DATE	AV. FRESHMEN 10 MIN. AREA	DATE	AV. SOPHOMORES 10 MIN. AREA
Class of 1928	Jan. 1925	21545 sq. mm.	Oct. 1925	18163 sq. mm.
Class of 1929	Oct. 1925	22829		
	Feb. 1926	22900	Oct. 1926	20450
Class of 1930	Oct. 1926	22999		
	Av. Freshmen area, 22568 sq. mm.		Av. Sophomore area, 19306 sq. mm.	

From the Table it is apparent that the stomachs of the 1928 class were rather narrow, but comparing Freshmen and Sophomore records, initial width is a function of the amount of stomach contents and is not particularly influenced by other conditions. Length and area however are affected by some factor other than mere size of meal. The reduction in height between Freshman and Sophomore stages is about 20 mm. The average area is reduced from 22600 to 19300 sq. mm. amounting almost to one-sixth of the latter figure. We have here a quite remarkable confirmation of the thesis presented in our first article, namely, the relatively small size of gastric shadow in Sophomores, after ingestion of a standard barium meal, when compared with the findings recorded for Freshmen.

GASTRIC DIMENSIONS AND PERIOD OF DIGESTION

Since the faint shadow of the empty stomach cannot be measured on the rare occasions when it can be seen in fluoroscopic examination, the nearest approach we can make to a comparison of dimensions in well-filled and approxi-

mately empty states is an examination of the dimensions taken from radiograms obtained respectively ten and sixty minutes after ingestion of the meal. These results are given in Table IV. The sixty-minute stomach is not empty. In a normal healthy subject we often find that a remnant of barium contents is still present in the most dependent portion of the pyloric vestibule even six hours after a meal. This is expelled immediately upon or very shortly after the appearance of a new meal in the stomach.

The successive classes of students are arranged as groups, and a careful examination of the respective reductions in dimension shows a progression in which the least reduction is exhibited throughout in the October Freshmen. The reduction becomes more marked in the January (or February) Freshmen and is at its greatest in the October Sophomores. Further, Group III as Freshmen gives a better showing than Group I as Freshmen. A factor which we have defined formerly as the group response is at work increasing the vigor of reduction in all dimensions, even width. If we assume that this reduction in dimension is due simply to emptying, we still have to cope with the problem of how it comes about that emptying is more vigorous in Sophomores than in Freshmen and also in successive groups of Freshmen. We shall see later that the explanation is probably not a simple one of emptying rate.

RELATION OF GASTRIC DIMENSIONS TO SIZE OF MEAL

In Tables III, IV are records of dimensions obtained from 16-ounce buttermilk meals. Table V gives records from 5-ounce sweet milk meals in addition to embodying the averages obtained from the two previous tables.

TABLE IV

RELATION OF STOMACH DIMENSIONS TO PERIOD OF DIGESTION

1. Width

		YEAR	AVERAGE 10 MIN. WIDTH	AVERAGE, REDUCTION FROM 10 TO 60 MIN.
Group I	Freshmen	Jan. 1925	71.1 mm.	15.1 mm.
	Sophomores	Oct. 1925	72.3	17.4
Group II	Freshmen	Oct. 1925	80.7	13.3
	Sophomores	Oct. 1926	83.1	24.3
Group III	Freshmen	Oct. 1926	81.1	17.7
			Total average reduction	17.6 mm.

2. Height

		YEAR	AVERAGE 10 MIN. HEIGHT	AV. REDUCTION 10 TO 60 MIN.
Group I	Freshmen	Jan. 1925	252.1 mm.	22.1 mm.
	Sophomores	Oct. 1925	230.6	20.8
Group II	Freshmen	Oct. 1925	245.4	10.0
	Sophomores	Oct. 1926	231.5	19.1
Group III	Freshmen	Oct. 1926	250.4	19.2
			Total average reduction	18.2 mm.

3. Area

		YEAR	AV. AREA 10 MIN.	AV. AREA 1 HR.	REDUCTION
Group I	Freshmen	Jan. 1925	21545 sq. mm.	17343 sq. mm.	4202 sq. mm.
	Sophomores	Oct. 1925	18163	13721	4442
Group II	Freshmen	Oct. 1925	22829	18913	3916
	Freshmen	Feb. 1926	22900	18077	4823
	Sophomores	Oct. 1926	20450	14690	5760
				Total average reduction	4629 sq. mm.

TABLE V

RELATION OF STOMACH DIMENSIONS TO SIZE OF MEAL

	16 OUNCE MEALS		5 OUNCE MEALS
	FRESHMEN	SOPHOMORES	SOPHOMORES
	10 MIN. AVERAGE	10 MIN. AVERAGE	10 MIN. AVERAGE
Width	77.6 mm.	77.7 mm.	51.4 mm.
Height	249.3	231.0	210.7
Area	22458.0 sq. mm.	19306.0 sq. mm.	12756.0 sq. mm.

The initial shadow width, which was 77.6 mm. on the average in the Freshman 16-ounce meal, is still the same (77.7 mm.) in the Sophomore meal of the same size. But it is only 51.4 mm. in the Sophomore 5-ounce average. This suggests again what we have already intimated, namely, that width bears initially a definite relation to stomach contents and is constant for a given size of meal.

We have shown that this is not true of height (Table III) and the reduction in height of shadow during the hour averages about 15 mm. for October Freshmen and 20 mm. for Sophomores (Table IV). The fluoroscopic records of these examinations usually state that at the end of the hour there appeared to remain about a quarter or rather less of the original contents. This was estimated on surface area of shadow only. The lateral fluoroscopic examination gives a very indefinite idea of anteroposterior dimension, though indeed this does not apparently change. Now the average reduction in width for Sophomores (Table IV) is about 21 mm. This subtracted from 78 mm. gives the figure 57 mm. which is strikingly near the new 5-ounce width, namely, 51.4 mm. Likewise the initial figure for Sophomore height reduced after an hour by 20 mm. (Table IV) gives a final figure of 210 mm. The actual comparable figure for the 5-ounce stomach is 211 mm.

In the problem of area we find a similar result. The average Sophomore stomach shadow of 19,300 sq. mm. is reduced by 5100 sq. mm. during one hour, and is hence brought to a final average of 14,200 sq. mm. Now the initial area of the 5-ounce shadow approaches 13,000 sq. mm.

It follows that our estimate of the amount of contents still present an hour after swallowing a 16-ounce meal is probably fairly accurate. The most instructive feature of these observations is that, worked out on the Sophomore stomach, all figures are quite logical and harmonious and their relationship to the actual contents seems assured. Hence as the Sophomore figures fall into line so perfectly there must be some influence, hitherto unrecognized, at work in the Freshmen records, throwing them into confusion. One might put it crudely by stating that the Freshman stomach is too large for its contents; but that this initial overrelaxation becomes progressively less marked in succeeding examinations of a single group, as it does also in the examination of successive groups. Naturally we do not seriously mean to put forward any such absurd explanation. But we would draw attention to the influence upon gastric tone of another factor, of psychologic or rather of central origin, which complicates the normal reflex gastric responses. In the various phases of our investigation we are constantly meeting the same phenomenon which baffles all our efforts to obtain a standard reaction pattern in the stomachs

of our Freshmen, but of which the influence is so greatly reduced in our Sophomore records that it is comparatively negligible. It is an inhibiting influence which limits the vigor and fullness of response in the untrained stomach.

THE VERTICAL DIMENSION OF THE MAGENBLASE

At this juncture we must return to the problem of vertical diameter of the stomach shadow. We have discussed rather fully the reduction in height of shadow which takes place during the hour following a meal and in the comparison of records taken from Freshmen and Sophomores. But we also must admit that this reduction finds no necessary or proportional counterpart in rise of greater curvature. For a long time we were at a loss to account for this. In the course of time we came to study the Magenblase itself and found that here also considerable change in vertical dimension takes place. When a meal is swallowed the elevation of the Magenblase can be clearly seen, the cupola of the diaphragm rising with it. The base of the Magenblase, which we have picked out as a stable level for measurement of width, however, remains constant in site. In some of our subjects we have found the vertical increase in Magenblase very marked, associated with extreme elevation of the diaphragmatic cupola. In all stomachs the reduction in Magenblase height goes on apace after some twenty minutes and at the end of an hour the reduction is quite pronounced. Many instances of reduction in gastric length were missed in our earlier work because our attention was focused on the greater curvature which scarcely changed in position, the contraction being almost confined to the fundic end. The slight respiratory distress which sometimes occurs during the first twenty minutes after a meal is probably associated with this phenomenon.

Vertical distension of the Magenblase can be observed on the fluoroscopic screen. It is evidently produced by a rapid accumulation of gas. In some subjects this occurs even before the actual swallowing of the barium meal. No matter whether it precedes, accompanies or follows the ingestion of the meal we have been quite unable to observe progress of gas down the esophagus. We also have looked in vain for gas entering the stomach through the pylorus. We are therefore forced to the conclusion that gas is formed in the Magenblase itself. And this phenomenon offers a problem for future elucidation. It is apparently analogous to the sudden accumulation of gas in the colonic flexures but is not so easily explained.

STABILIZATION OF THE STOMACH

At his first roentgenoscopic examination the Freshman shows certain signs of distress such as pallor, flushing, dryness of mouth, difficulty in speech, a cold clammy skin, perspiring palms, and jerky nervous physical movements. The manifestation of this distress varies in range and in degree, but with these outward signs we may expect others, more obscure, exhibited in the viscera. We have indeed already given reasons for the conclusion that such manifestations are to be found in the alimentary tract. The Sophomore, with his growing experience of medical training, rarely exhibits outward indica-

tions of distress. Nevertheless, even in him, an unexpected stimulus produces an immediate effect which, despite rapid recovery, points toward the condition characteristically found and more pronounced in the Freshman.

Recognizing then the disturbing effect of these emotional factors it has been possible to develop a method of training and a technic of observation by which the influence of these factors is minimized. Repeated examinations of the same subject are made under carefully controlled conditions and a rigid discipline. Not only is the subject under constant scrutiny but the discipline extends to examiners and observers who must work in perfect harmony. If even the slightest antagonism develops, of which the subject himself is entirely unconscious, it will make its effect evident in the experimental result.

A subject of even temperament exhibits a stomach in which there are but slight fluctuations in position and activity at successive examinations. We have called this equable type a tolerant stomach.

Few individuals exhibit a tolerant stomach at the first examination. In nearly all the stomach shadow controlled by measurements at later examinations, is long and broad. The peristalsis is feeble and often temporarily inhibited. Reduction in size of shadow and increased vigor of peristalsis are shown at successive examinations until, after practice, the responses to a constant stimulus are stabilized both in dimensions and in motility. Such a stomach we hold to exhibit evidence of the distress complex. According as the behavior pattern is further removed from the modal response, we term the degree, disquiet, apprehensive or distressed. In fear, the response reaches its culmination: there is then great exaggeration of shadow dimensions with complete inhibition of peristalsis. We have reason to believe that fatigue also induces, though in less degree, a somewhat similar behavior pattern.

On the other hand a subject, even though trained, may present the picture of an especially active stomach. The shadow dimensions are small and the peristalsis unusually active. A small degree of this aberrancy is indicated by the term anxious, a greater degree by the adjective embarrassed. Both are included in the category of the anxiety complex to distinguish them from the classification of the distress complex outlined in the foregoing paragraph.

Any stomach may on occasion show a flocculent condition of the contained barium. We have noted the appearance of this phenomenon in conditions of temporary agitation. This is much more likely to be associated with the anxiety complex than with the distress complex.

It is our purpose in succeeding communications to develop the themes here indicated in broad outline. On the one hand we intend to present our evidence for the existence of disturbing emotional factors, a subject of great importance for psychologic study inasmuch as the indicator is not only beyond the power of voluntary control but in addition, is quite incapable of being dragged up over the threshold of consciousness. On the other hand, with the emotional factor controlled and minimized, we plan to present the evidence for definite behavior patterns in gastric response to particular and constant stimuli.

SUMMARY

In a former article we put forward the thesis that intrusion of impulses of central (psychologic) origin complicates the reaction patterns of untrained alimentary tracts and is in part responsible for the variation in response characteristic of previous investigations on the subject. In this essay we have subjected the above thesis to a quantitative analysis by such methods as are possible at this time.

From fortunate glimpses of the empty stomach and from a study of the phenomena of filling we have noted the extraordinary facility with which the stomach distends laterally, this distension being apparently induced by a sudden outpouring of juice. Further experiments along these lines will be recorded later.

Owing to the relatively slight change in anteroposterior diameter, dimensions of the barium shadow give a fair degree of probable accuracy to estimates of amount of contents. But while the figures hang well together and give intrinsic evidence of reliability in Sophomores, it is quite impossible to bring the Freshmen results into line. This is due to the unstable character of Freshmen reaction patterns influenced by central impulses which are normally at a minimum in Sophomores.

Especially noteworthy in the gastric reaction pattern is the fluctuation in vertical diameter of the Magenblase.

By suitable training the reaction pattern of gastric response can be stabilized and the human stomach thus prepared for experiments comparable to those of the laboratory.

ABSTRACT

The thesis that intrusion of impulses of central origin complicates the gastric reaction patterns of untrained subjects is subjected to a quantitative analysis. It is shown that by training the subjects, gastric reaction patterns can be stabilized and the human stomach prepared for experiments of an exact nature comparable to those of the laboratory.

LABORATORY METHODS

RESPIRATORY CABINET FOR LABORATORY ANIMALS*

By J. J. R. Macleod, Assisted by Miss N. R. Hearn, and F. L. Robinson, Toronto

RESPIRATORY CABINET

General Directions.—During the past few years numerous observations have been made in this laboratory[1] on the respiratory exchange of laboratory animals by means of an apparatus devised according to the principles described by Benedict and Tompkins[2] in 1916. This apparatus has been constructed of suitable size for dogs weighing up to 14 kg. It is also perfectly accurate for small animals. It consists in general of an air-tight metal box, with a movable lid, ventilated by an air current which passes in, through tubing, at the top of the box and is removed from the bottom, the current being maintained by a rotary blower inserted in the outgoing tubing. The air then enters a system of absorbing bottles for the removal of water and CO_2, and after being again moistened it is returned to the box. On the lid is a Krogh spirometer, the movements of which are inscribed on a smoked surface. As the volume of air in the system becomes less, owing to the absorption of CO_2, the descent of the spirometer makes contact in an electric circuit which thereby operates an electromagnet so that a clamp, which is compressing a thin rubber tube through which oxygen is passing, is opened and O_2 flows from a tank at uniform low pressure, through a Bohr meter, into the inlet tube of the apparatus.

The CO_2 expired by the animal is measured by weighing the absorption bottles, and the oxygen, by the volume recorded on the meter. Correction is also made for variations in volume of the air in the chamber due to changes in temperature and barometric pressure. For this purpose several thermometers, reading to 0.05° C., are inserted in the chamber, one of which extends to its bottom and three are near the top. Two of the latter are close together, one of them being a wet-bulb thermometer, and a small fan is inserted through the walls of the chamber so as to direct a constant air current onto it. This serves as a psychrometer for measurement of the aqueous tension.

For the purpose of testing the accuracy of the apparatus, a measured quantity of alcohol is burned in a cylinder inserted in the system of tubing.

The general plan of the apparatus is shown in Fig. 1,† and a photograph of it is also shown in Fig. 3.

*From the Physiological Laboratory, University of Toronto, Canada.
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†The balance used for weighing the absorption bottles was supplied by Aug. Sauter, Ebingen, Würtemberg, Germany. (Precision Balance No. 7, 10 kg.)

DETAILED DESCRIPTION

1. *Respiratory Chamber (Fig. 1).*—This is made of galvanized iron, 22 gauge. It is double walled, the dimensions of the outer casing being 47 by 89 by 52 cm. and those of the inner one, 38 by 80 by 45 cm. The space between the two walls is filled with water. At a distance of 5 cm. from the top, the space between the two walls is bridged across so as to form a water trough into which the lid of the box fits. The water in the space below is kept stirred up by means of an air current delivered through a perforated tube at the lower end of the box. The lid, which can be raised by a pulley, measures 43 by 86 cm. and is provided with a flange 5 cm. deep which fits into the water trough. It also has a glass window, *W*, 30 by 30 cm., and smaller openings for the insertion of the thermometers, *T*. One of these, at a corner of the lid, is for a long-stemmed thermometer which reaches to the lower end of the box (not shown in figure). The stem of this thermometer is protected by a metal tube, slotted along one side, the slot being covered by stout wire gauze. Two other thermometers are placed about the center of the box and their bulbs project just below the lid. One of these is kept moist by means of a wick which dips into a small bottle filled with water, the pair therefore serving for the measurement of humidity. On one side of the box is an opening through which, with suitable packing boxes, passes the shaft of a small fan, *K*, driven by a belt coming from the blower-motor. Another thermometer is placed at the corner of the lid farthest from the long thermometer.

The Krogh spirometer, *S*, is directly mounted on the lid and is connected with the chamber by a ¾ inch tube. This spirometer has a capacity of 1800 c.c. and its lid, which is carefully counterpoised, is connected with a long lever which inscribes the movements on a travelling surface, and has connected with it at right angles an arm which passes down to a mercury cup, to complete the electric circuit referred to above. There is sufficient give in the walls of the box so that any movements of the animal are distinctly recorded by the spirometer. The outer case of the spirometer is made of copper and the lid of very thin zinc (16 gauge), the metal surfaces being carefully covered with shellac.

We have found it of advantage to have the lid heat-insulated, by covering it with a deep layer of broken cork held in position by flanges along the edges. For raising and lowering the lid it is necessary to empty the water out of the trough, and this is done by means of a ¾ inch pipe connected at one corner *L*. The water in the water jacket may similarly be evacuated by means of a ¾ inch pipe at the lower corner *L'*. Finally two narrow tubes are inserted, one through the lower part of the box and the other through the lid for the purpose of removing samples of air for analysis.

Resting on the floor of the chamber is a flanged tray, which slopes slightly toward one end where there is a pipe connected outside, by means of rubber tubing, to a flask for the collection of urine. An inner tray of wire mesh prevents the animal from resting directly on the sloping tray. These trays are easily taken out for washing, etc.

2. *Absorbing System.*—The air is taken from the lower end of the box by a ¾ inch pipe which branches inside the water jacket so that its openings are

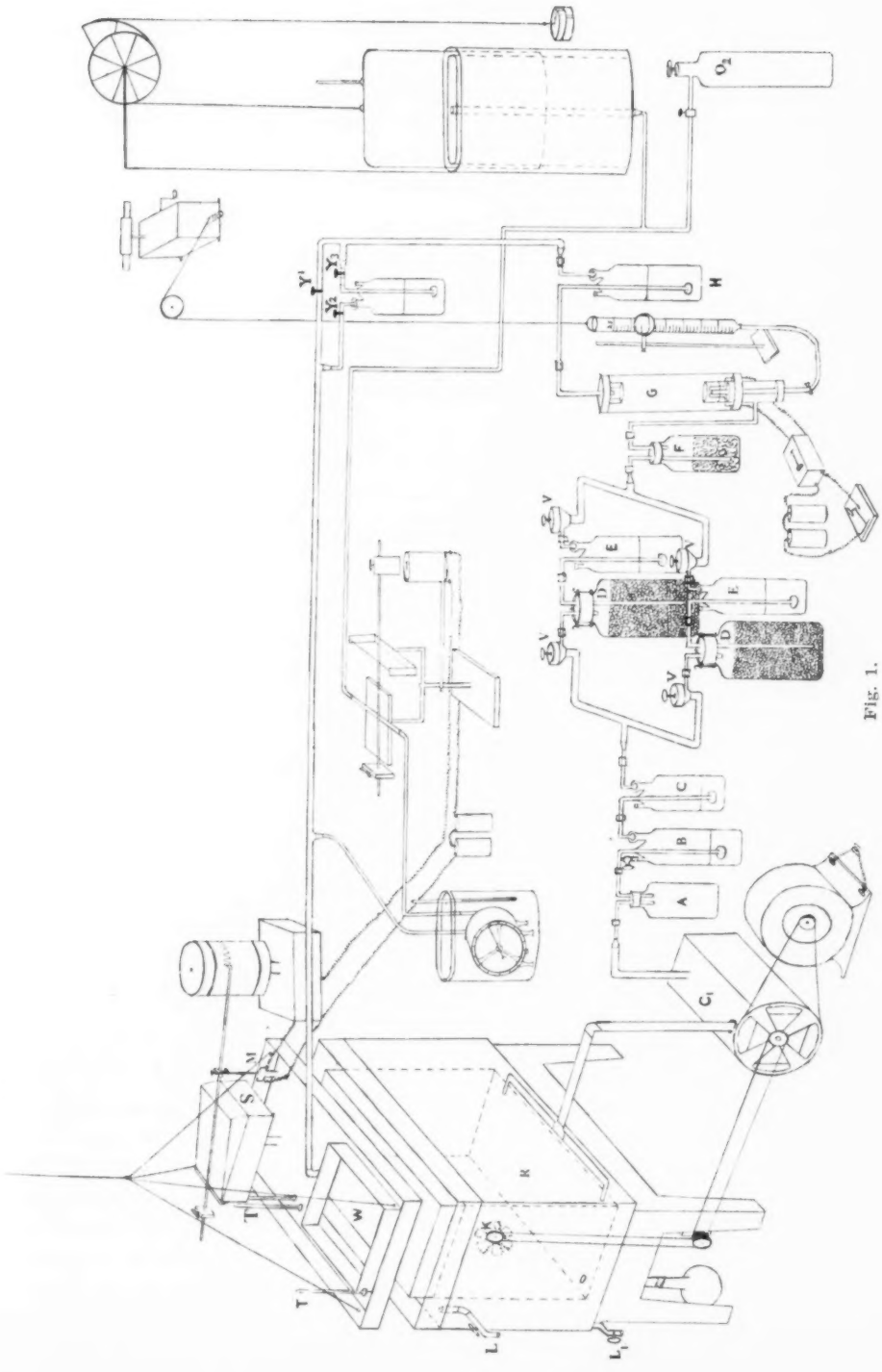

Fig. 1.

at the lower corners of the box. Immediately after leaving the box the pipe is joined by a brass coupling with the negative side of a Crowell blower, *Cr.* (Crowell Mnfg. Co., Brooklyn, N. Y.: No. OD rotary compressor), which is placed in an iron box filled with oil. A suitable ventilation, about 15 liters per minute, is obtained when the motor connected with the blower is rotating at a speed of 110 revolutions per minute.

The positive side of the blower is connected with a wide-mouthed bottle, *A*, of about 1 liter capacity, to serve as a trap preventing any sulphuric acid being accidentally sucked back into the pump. The pipe leading from this bottle is coupled with a large Williams bottle, *B* (of 2 liters capacity), containing 750 c.c. concentrated sulphuric acid. In order to make the union between glass and metal air-tight, the tubes of the Williams bottles are fitted with brass female couplings of standard, ¾ inch size, by placing a piece of rubber tubing over the glass tube and then turning the coupling so that it fits snugly on this tube. If any leakage should occur this may be stopped by Chatterton's cement. The third tube of this Williams bottle, *B*, is provided with a glass stopcock which is used for adjusting the pressure of the system at the start of an observation. A second Williams bottle, *C* (of 1 liter capacity), containing 500 c.c. sulphuric acid, follows and its outlet tube is connected, through a coupling, and a piece of wide rubber tubing with the T-piece which carries the valves, *V*, the construction of which will be described in detail below.

After passing through either valve the air is led to the CO_2-absorbing bottle, *D*, which can be quickly detached by unloosening the couplings connecting it with the valve and the Williams bottle containing sulphuric acid, *E*, which next follows. This connects with a second pair of valves and a T-piece, the central tube of which is coupled to a pipe leading to the bottom of a wide-mouthed bottle, *F*, which is filled with cotton to collect any sulphuric acid spray which may come over. The outlet tube of this bottle enters the lower end of a glass cylinder, *G*, of 7 cm. diameter and 28½ cm. long for burning alcohol, the upper end being connected, through suitable couplings and a short piece of rubber tubing, with a large-sized Williams bottle, *H*, which contains water for the purpose of moistening the air before it returns to the chamber which it does through a pipe opening in the center of the lid. On this pipe is a side tube which delivers oxygen into the system through a Bohr meter at a rate regulated by the electromagnetic valve. In the drawing the tubing near the absorption bottles is represented as being of smaller diameter than elsewhere. This is not actually the case.

Valves: Since some trouble was experienced in obtaining, on the market, valves that were satisfactorily air-proof, special ones were designed, as shown roughly in Fig. 2. Each consists of a cylindrical portion, 2 cm. high and 6 cm. in diameter, and a funnel-shaped portion narrowing down to an opening of 2 cm. through a distance of 4 cm. and provided with a side-tube of standard (¾ inch) size. Between the cylindrical and funnel-shaped portions is an internal ledge 5 mm. wide and 1 mm. in thickness. This part of the valve is cast in brass. The cylindrical portion is threaded on the inside (32 to the inch) to take the flange of a brass cover, and through the center of the cover passes

a spindle, threaded 8 to the inch. This terminates in a handle above and is rounded into a small ball below. A diaphragm, 6 cm. in diameter, cut out of rubber, 5 mm. thick rests on the ledge on to which it is pressed down by the flange of the lid, a cast washer 5 mm. thick being interposed between. Through the center of the diaphragm passes a brass rod ($\frac{3}{16}$ inch) ending above in a socket which fits the ball-shaped end of the spindle. This rod is threaded for a nut which is screwed up against the rubber diaphragm and socket. Its lower end passes through a rubber stopper which is held in position on the rod by two nuts and is shaped so as to close the lower opening. The valves are connected up as shown in the diagram of Fig. 1. These valves have given very satisfactory service and are completely leak-proof, provided the rubber diaphragm and stoppers are replaced about once every month during daily use of the apparatus.

The CO_2-absorbing bottles: Wide-mouthed glass bottles of 1 liter capacity furnished with a brass cover carrying the inlet and outlet tubes have proved much more satisfactory than those usually employed. A circular cast brass

Fig. 2.

plate (of the same diameter as the mouth of the bottle) has two brass tubes of standard size passing through, and brazed on to it. To one of these is soldered a lead tube long enough to extend to the bottom of the bottle. The brass lid is clamped down, in the manner shown in Fig. 1, to a brass collar which is cemented on to the neck of the bottle by means of aquarium cement. Wilson soda lime, 4-8 mesh (supplied by Warren Collins, Boston, Mass.), is used to absorb the CO_2. One kilo of this absorbs from 60 to 70 gm. of CO_2 and when there is doubt as to whether absorption is proceeding efficiently, the air current is deflected through a Williams flask containing baryta water, by suitable adjustment of the clamps Y_1, Y_2 and Y_3 (Fig. 1).

Further details of the apparatus are sufficiently given in the sketch plan and photograph (Fig. 3).

The calculation of the results is carried out exactly as described by Benedict and his coworkers. In the observations made by us, the CO_2 bottles have been weighed at intervals of an hour when also the meter and the thermometers were read. The total duration of each observation was usually eight hours. At periods, samples of air were removed from the chamber for analysis in a Haldane gas burette, and if any considerable deviation was found

in the percentage of CO_2 the accuracy of the respiratory observation was placed in doubt. Experience soon showed, however, that little error could be incurred on account of an unequal rate of absorption of CO_2, especially when the animal was quiet, so that this check came latterly to be little used. Chance of error on account of the omission was guarded against, by placing the animal in the chamber with absorption apparatus in position for at least one-half

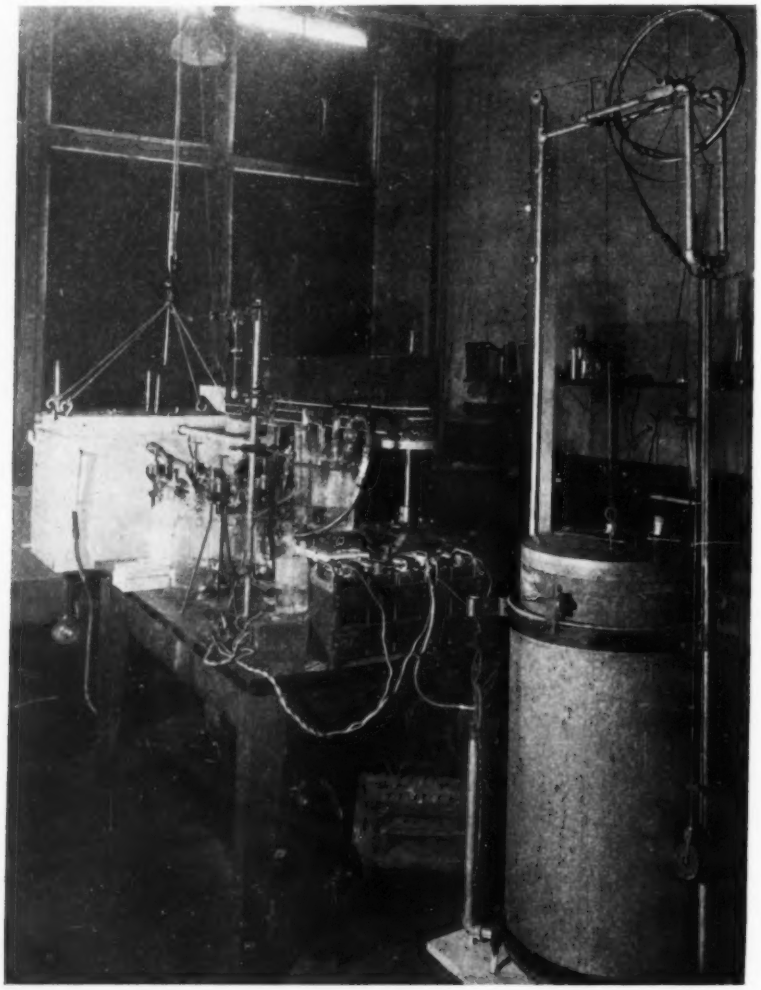

Fig. 3.

hour before actually using weighed bottles, or reading the meter. During this preliminary period, also, the volume of the air in the system was allowed to become constant, its expansion, due to the heat given off by the animal, being corrected by opening the escape value on *B*. Most of the animals after a short experience in the chamber became quiet and indeed often slept throughout the observation. Their movements were recorded on the smoked drum, and if these proved to be considerable the result was put in question since, under

such conditions, CO_2 might be produced at a rate in excess of that at which it was being absorbed by the soda lime. However, since the observation was continued for several hours and the animal usually was quietest toward the end, any error due to this cause was eliminated in the average result for the day.

Accuracy of apparatus: This was tested in two ways: (1) By seeing whether the volume of air remained unchanged without having an animal in the box, but with the pump running. This was usually tested for several hourly periods and was found to be a reliable method for detecting leaks. (2) By burning a known volume of alcohol in the glass cylinder placed in series in the absorption system, as shown in Fig. 1. The burner for the alcohol and the manner of feeding it with alcohol from a burette are also shown, and it may be said that after the correct rate for raising the feeding burette has been determined by adjusting the speed of the clock by which the thread supporting it is wound (see Fig. 1), there is no technical difficulty in applying this check. At first we attempted to place the alcohol burner in the respiratory cabinet, but the results were unsatisfactory because of the sudden changes in volume of air occurring, not only when the flame was lighted but during its burning. This also was usually more or less irregular, probably owing to variability in the oxygen supply in the immediate neighborhood of the flame. This irregularity meant that combustion was not always complete and a smell of unburned alcohol could often be detected when the lid was removed from the chamber. Moreover, while in the chamber the alcohol flame often went out, which seldom occurs when the cylinder is used. If it does go out, the flame is readily started again by the sparkling of a high tension electric current. Another source of error incurred with the flame in the chamber is owing to drying of the air. This is avoided by the cylinder method because of the moistener, *H*.

Table I gives the results of some alcohol tests run during the spring of the present year, and they show satisfactory agreement with regard to both O_2 consumption and R.Q.

Taking all observations together there is an excess of 71 c.c., or 0.26 per cent of CO_2 and an excess of 304 c.c., or 0.76 per cent of O_2, while the average R.Q. is 0.664, the theoretical being 0.667. Since the error in both CO_2 and O_2

TABLE I

DATE 1928	DURATION OF PERIOD			ALCOHOL BURNED	CO_2 PRODUCED		O_2 USED		R.Q.
				93.44%	FOUND	THEOR.	FOUND	THEOR.	THEOR. 0.667
	hr.	min.	sec.	at 20° C.	c.c.	c.c.	c.c.	c.c.	
Mar. 1	1	54	0	3 c.c.	2274	2252	3397	3370	0.669
" 12	1	35	0	6 c.c.	4490	4504	6711	6740	0.669
" 15	0	40	0	3 c.c.	2124	2162	3181	3244	0.668
" 27	1	01	30	3 c.c.	2160	2175	3273	3264	0.660
" 27	0	59	30	3 c.c.	2137	2198	3243	3298	0.659
Apr. 3	0	58	30	3 c.c.	2259	2193	3394	3291	0.666
May 11	1	07	0	3 c.c.	2193	2194	3334	3291	0.659
" 11	1	19	0	3 c.c.	2198	2194	3333	3291	0.659
" 16	0	40	0	3 c.c.	2173	2198	3256	3298	0.667
" 30	1	02	0	3 c.c.	2264	2198	3380	3298	0.670
" 30	1	10	0	3 c.c.	2325	2198	3485	3298	0.667
Total				36 c.c.	26537	26466	39987	39683	Av. 0.664

measurements is sometimes *plus* and sometimes *minus* in the different experiments, it must be incurred, not because of any slight leak in the apparatus, but owing to inaccuracy in weighing the CO_2 absorbers, and probably also because we have to depend on wet- and dry-bulb thermometers for determination of the water vapor. In any case, the error is of little consequence in the type of observation for which the apparatus has been used. Besides these special tests for accuracy any error due to leakage, or other cause, could usually be detected in the course of a series of observations by watching the behavior of the R.Q. In depancreatized dogs this is remarkably constant for at least the first week after withdrawal of insulin and food, so that any fault in the technic was usually revealed by an abnormal quotient during the first hour of the observation.

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STABILITY OF DESICCATED BEEF HEART MUSCLE POWDER FOR PREPARATION OF ANTIGENS FOR COMPLEMENT-FIXATION TESTS IN SYPHILIS, WITH SPECIAL REFERENCE TO THE KOLMER METHOD*

By Robert A. Kilduffe,† A.M., M.D., and Betty Shifman, Atlantic City, N. J.

ONE of us (R. A. K.) has previously reported[1] that commercially prepared desiccated beef heart powder‡ was quite stabile and suitable after the expiration of eleven months for the preparation of antigens satisfactory for the conduct of complement-fixation tests in syphilis by Kolmer's method.

Recently, through the courtesy of Mr. H. G. Dunham of the Digestive Ferments Co., we have been able to test beef heart powders varying in age from sixteen to sixty-four months with the results shown below.

Both the old and new titrations were carried out in exact accordance with the method described by Kolmer.[2]

It is of interest to note that the powders were kept at room temperature and without special precautions in cork-stoppered bottles.

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*From the Laboratories of the Atlantic City Hospital.
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†Director, Laboratories, Atlantic City Hospital.
‡Digestive Ferments Co.

NO.	DATE POWDER MADE	ORIGINAL TITER	NEW TITER	AGE OF POWDER WHEN NEW ANTIGEN MADE
11631	5/ 8/23	Hem. not in 1:4 Antic. 1:6 Antig. 1:2000	Hem. not in 1:4 Antic. 1:20 Antig. 1:4000	64 months
12145	11/21/23	Hem. not in 1:4 Antic. 1:6 Antig. 1:2400	Hem. not in 1:4 Antic. 1:20 Antig. 1:3200	59 months
12692	6/23/24	Hem. not in 1:4 Antic. 1:6 Antig. 1:3200	Hem. not in 1:4 Antic. 1:12 Antig. 1:1200	52 months
13564	2/16/25	Hem. not in 1:4 Antic. 1:6 Antig. 1:4000	Hem. 1:6 Antic. 1:32 Antig. 1:4000	44 months
14375	9/ 4/25	Hem. not in 1:4 Antic. 1:16 Antig. 1:4000	Hem. 1:4 Antic. 1:32 Antig. 1:4000	37 months
17019	6/24/27	Hem. not in 1:4 Antic. 1:8 Antig. 1:3200	Hem. not in 1:4 Antic. 1:16 Antig. 1:4000	16 months

The conclusions formed from the previous investigation may be again repeated but with added emphasis, namely:

1. Under ordinary conditions of storage desiccated beef heart powder is quite stabile, satisfactory antigens having been prepared from it after five years and four months.

2. The only change encountered was a slight increase in the anticomplementary value, in no case sufficient to interfere with the antigenic efficiency of the extract.

3. Beef heart muscle powder may be prepared commercially and stored in large quantities for at least five years without fear of undue deterioration.

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METABOLISM MASK FOR DOGS*

By Rebekah Gibbons, Omaha, Nebraska

A MASK suitable for connecting dogs to any common type of metabolism apparatus has been devised. It has the following advantages:

1. The animal's eyes need not be covered during the tests. The operator may thus more easily assure himself that the animal is awake.

2. The animal is only very slightly disturbed in adjusting the mask.

3. The rebreathing volume is very small.

4. The pressure necessary to assure an air-tight connection is borne by tissues supported by jaw and cheek bones. Circulation and respiration are not interfered with. The greater comfort from this mask may be determined subjectively by encircling one's neck firmly but lightly with the hands and comparing the mild discomfort resulting, with the lack of such discomfort when considerable pressure is exerted for the width of a single finger to the bony parts of the nose and the cheek and jaw bones.

5. There is no chance of the mask tearing at an inconvenient moment as is the case when the animal's mouth parts are forced into a small hole cut in sheet rubber.

6. The animal's mouth and lips may be comfortably arranged before the mask is tightened. Creases in the skin, even with dogs having redundant lips, may be more easily eliminated.

7. The flexibility of the mask allows a slight pulsation during both inspiration and expiration, thereby buffering pressure changes in the mask and further helping to obviate leaks.

The flexible mask constructed entirely of rubber is shown in the illustration.

DESCRIPTION OF THE MASK

The mask is cylindrical in shape and carries in one of the bases a piece of rubber tubing of suitable size and length by which it may be connected to the respiration apparatus. After it has been comfortably adjusted with the animal's lips pushed far forward and the open portion of the mask pulled well up toward the eyes, it is made air-tight by wrapping a strong rubber band approximately one-half inch wide twice around the mask and fastening it with a hemostat. On the first turn the band is stretched as much as is possible without causing puckers in the rubber of the mask. On the second turn the band is stretched as tightly as is consistent with the comfort of the animal. Care should be exercised that the animal's lips are in front of the band. If the mask is correctly adjusted well up toward the eyes, the considerable pressure of the band is almost entirely borne by tissues well supported by cheek and jaw bones. Ease of respiration is not interfered with nor is the circulation hampered, and the animals submit without muscle tension to considerable stretching of the rubber band. As originally conceived, the mask was cone shaped to obviate

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rebreathing in so far as possible, but the pressure on the soft tissues toward the nostril was great enough to cause discomfort. This is not the case with the cylindrical mask which when in place assumes the shape of a broadly truncated cone.

THE USE OF THE MASK

With moderate tension of the band an air-tight connection can be secured on dogs with short, soft hair without showing leaks. For dogs with wiry hair such as Airedales, the band must be considerably tighter but satisfactory tests may be easily secured. The omission of shaving saves considerable time as well as the annoyance of an occasional skin wound or other abrasion which sometimes interferes with a satisfactory determination at a time when it is desirable to test the animal.

TESTING THE MASK

The tracings which have been reproduced were made from unshaved dogs using a Benedict portable respiration apparatus. A clockwork kymograph was used for the graphic records. The slope of the respiratory tracing therefore

Fig. 1.—Cylindrical rubber mask for attaching dogs to metabolism apparatus. Also the heavy rubber band and hemostat used to produce an air-tight connection.

becomes a measure of the intensity of the metabolism. The arrow on the tracing indicates the removal of a 40 gm. weight from the spirometer bell. Tracing I shows a typical satisfactory test. The fall of the bell for the "weighted" and "unweighted" portions of the test period was the same. Tracing II shows the effect of a small leak resulting from insufficient tightening of the rubber band. The slope of the line indicating the fall of the spirometer bell is sharper for the "weighted" than the "unweighted" part of the test period.

DIRECTIONS FOR MAKING THE MASK

To make a mask such as the one here described a rectangular section is cut from sheet rubber. One dimension equals 7 mm. less than the circumference of the dog's muzzle just below the eyes. This provides for a 7 mm. lap making the finished mask smaller in circumference than the part of the dog's face to which it is to be applied. This allows enough stretch so that it will fit snugly and hold the angle of the lips well forward, but at the same time permits comfortable adjustment and easy breathing. The other dimension equals the distance from the position which the top of the mask will occupy when in place on the dog, and the tip of the animal's nose plus 30 mm. This provides for

20 mm. between the end of the dog's nose and the outlet tube in the finished mask and a 10 mm. allowance to turn up and cement onto the base. A circular section with a circumference equal to the long dimension of the rectangular piece less the lap is also cut for the base.

The ends of the rectangular piece should be lapped and vulcanized. Care should be exercised that the cushion rubber receives sufficient pressure so that there is no ridge at the seam. A better turn can be made over the base if the cushion rubber is cut an inch shorter than the mask rubber at this edge. There is no strain on it and the cement will hold without reinforcement.

The lower edge of the resulting tube is notched for turning onto the base. The V-shaped pieces removed being 10 mm. deep and a trifle wider than those

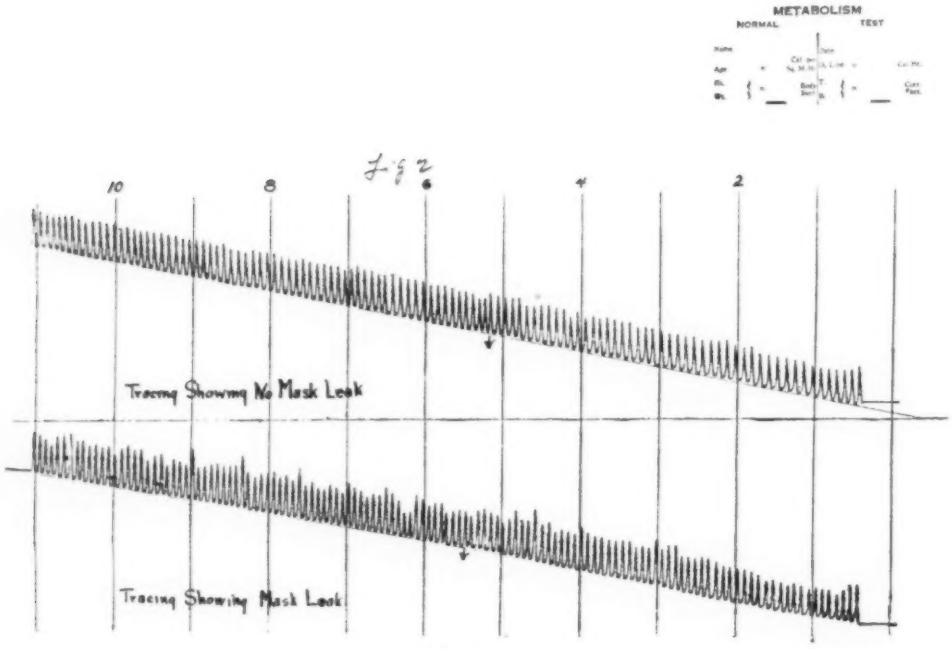

Fig. 2.

which are left. The V's which make up the saw-toothed edge should not be over 7 mm. wide if a smooth turn is to be made.

The mask is most easily put together if a bottle or can of the same diameter as that of the mask can be found. The notched tubular part is slipped over the bottle or can to within 3 mm. of the notched edge and the cement applied. Even pressure can now easily be exerted in cementing it onto the circular base. It may either be vulcanized or cold cemented. This part of the mask is not submitted to stretching so that vulcanizing is not necessary and is probably undesirable, for this part is awkward to vulcanize and occasionally an overcured spot results.

After the completed cylinder has been tested for air-tightness a small circular hole about 5 or 6 mm. in diameter is cut in the center of the base, and the large tubing by which the mask is attached to the apparatus is introduced and cemented into place.

THE DETECTION OF BLOOD BY MEANS OF BENZIDINE DIHYDROCHLORIDE*

By S. L. Leiboff, M.A., New York City

AMONG the various tests for the detection of blood in biologic materials, the guaiac and the benzidine tests are the most widely used. The guaiac is less sensitive and less reliable than the benzidine test. This is due chiefly to the fact that gum guaiac is an impure substance of indefinite composition. White,[1] who has studied a large number of cases with gastric ulcer, found that guaiac often failed to detect blood in feces while benzidine detected blood in all these cases. However, the test with benzidine must be carefully adjusted, as benzidine, particularly when applied to feces, may be too sensitive and give false positive results. Meat, fish, iodides, and iron salts give positive results. After eating meat, a positive test may be obtained with benzidine for three days after the meal.

Lyle and Curtman[2] prefer guaiaconic acid for the detection of blood in feces, since it is not as sensitive as benzidine and is more sensitive than guaiac. They describe a method for the preparation of this reagent.

Ruttan and Hardisty[3] found that acetic acid solutions of benzidine rapidly deteriorate in sensitivity toward blood, thus necessitating the use of freshly prepared solutions. They recommend the use of ortho-tolidin, which is benzidine containing two methyl groups.

Lyle, Curtman, and Marshall[4] found a number of factors to influence the accuracy of the benzidine test. They found that alcoholic solutions were not reliable, as positive tests were obtained with controls containing no blood. The ratio of the reagents used in the test must be properly adjusted. This is particularly true of the acetic acid and the hydrogen peroxide. The acetic acid serves merely to keep the benzidine in solution. An excess of acetic acid diminishes the delicacy of the test by increasing the speed with which the color fades. The purpose of the hydrogen peroxide is to supply oxygen. When too little hydrogen peroxide is used very little color develops, while an excess destroys the catalytic power of the blood and reacts with the benzidine itself.

The purpose of this paper is to eliminate the objectionable reagents; to prepare a stable benzidine solution; and to standardize the technic as to the proper ratios of the reagents used.

Benzidine combines easily with a great many substances and produces many highly colored compounds. It is easily oxidized to form colored substances, and upon this fact is based its use for the detection of blood; the peroxidases in the blood break up the H_2O_2 into water and liberate free oxygen which in turn oxidizes the benzidine producing the blue color.

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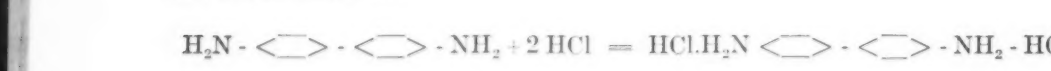
A number of compounds of benzidine have been prepared and tested as to their applicability for the detection of blood. Benzidine dihydrochloride was found very suitable for this purpose. It is soluble in water thus obviating the necessity of using acetic acid; it is quite stable (will last for at least three months without deterioration), thus obviating the necessity of using fresh solutions; it may be prepared in a very pure state.

Commercial preparations of benzidine dihydrochloride are very expensive and cannot be obtained in a pure state. It may be prepared in the laboratory by the following simple method:

PREPARATION OF BENZIDINE DIHYDROCHLORIDE

Place 20 gm. of crude benzidine base in a porcelain or glass mortar (400 to 500 c.c. capacity) and grind to a very fine powder with a pestle. Add about 150 c.c. of approximately normal HCl, grind up well with the pestle, let stand for about ten minutes to allow most of the sediment to settle, and pour the supernatant fluid into a liter beaker. Repeat this process three times more with 150 c.c. portions of normal HCl, and finally wash the sediment in the mortar four times with 50 c.c. portions of distilled water. The combined washings will now contain about 800 c.c. of fluid. The residue remaining in the mortar is discarded.

The combined washings in the beaker contain benzidine dihydrochloride in solution and a great deal of impurities, some in solution and some in suspension which give the solution a dark brown muddy color. The following reaction is involved:



Filter through filter paper into a two liter flask. Do not use suction as the sediment will pass through the filter paper. To the filtrate add about 10 gm. of animal charcoal, shake for a few minutes and filter through a clean filter paper. The solution will now be clear but quite brown in color. Shake again with another portion of charcoal and again filter. Repeat this process until the filtrate is perfectly colorless. It usually takes from four to five treatments with charcoal to obtain this, depending upon the purity of the benzidine base.

It is best not to interrupt the process and to continue until the finished product is obtained, for if the benzidine dihydrochloride is left too long in contact with the charcoal, the benzidine dihydrochloride itself will be adsorbed by the charcoal and will not pass through the filter paper. This is particularly true when left in a cold place. During no stage of the procedure should heat be applied, as heat will destroy the benzidine dihydrochloride.

The clear filtrate which now contains about 600 c.c. is placed in a liter beaker and 200 c.c. of concentrated HCl (Sp. Gr.=1.19) is added. A copious precipitate of beautiful white crystals of benzidine dihydrochloride is produced immediately.

Pour the suspended crystals on a glass filter (Buchner, Jena Glass, No. 8509, Will Corporation, Rochester, New York) and filter with suction. Trans-

fer the remaining crystals from the beaker to the filter by washing down with 50 per cent HCl from a wash bottle, washing down the sides of the beaker with a fine stream. Dry by suction as completely as possible.

The crystals must be freed from water and from free HCl. This is accomplished in the following manner: wash the crystals twice with 25 c.c. portions of 95 per cent alcohol, sucking through the alcohol completely before adding the second portion, and wash four times with 25 c.c. portions of ether, and suck dry as completely as possible. The crystals are now collected on a watch glass and placed in a desiccator over soda lime. In a few days the crystals are perfectly dry and devoid of free HCl. The crystals are placed in a brown bottle which is tightly stoppered.

Reagents Necessary for the Performance of the Test.—(1) One per cent benzidine dihydrochloride solution. This dissolves in water very slowly. Do not use heat to hasten solution, but shake at intervals for about two hours and filter into a dark brown bottle. It will keep for at least three months without any deterioration. (2) Three per cent hydrogen peroxide. The commercial 3 per cent hydrogen peroxide is not very suitable. Dilute 30 per cent "superoxol" 1 to 10.

DETECTION OF BLOOD IN URINE

One or more c.c. of urine is placed in a clean test tube and diluted with distilled water to about 5 c.c. One c.c. of benzidine solution and one c.c. of 3 per cent hydrogen peroxide are added. A blue color will develop in the presence of blood. The rapidity with which the color will develop will depend chiefly upon the amount of blood present; it usually takes from one to ten seconds.

When the amount of blood is very small, it is best to centrifuge the urine and after pouring off the supernatant fluid, the sediment is placed on a glass slide and one drop of benzidine solution and one drop of hydrogen peroxide are added and the contents mixed by tilting the slide.

The hydrogen peroxide must always be added last.

DETECTION OF BLOOD IN FECES

About one gram of feces is mixed well in a test tube with 5 c.c. of distilled water and brought to a boil over a flame, and filtered. The filtrate is collected in a clean test tube and cooled. One c.c. each of benzidine solution and hydrogen peroxide are added.

DISCUSSION

Since the sensitivity of the test is greatly influenced by the relative concentrations of the reagents, tests were performed with varying amounts of benzidine and hydrogen peroxide and the proper proportions were thus determined. Nine tubes were set up containing the same amount of blood and hydrogen peroxide each, but varying amounts of benzidine. The results are shown in Table I.

The same experiment was repeated using larger amounts of hydrogen peroxide. In this case the color increased with the concentration of benzidine;

the higher the concentration the more color developed. This is shown in Table II.

The blue color fades rapidly depending upon the amount of hydrogen peroxide; the more hydrogen peroxide used the quicker the color fades. Thus the tubes in Table I, where only a half c.c. of hydrogen peroxide was used began to fade within ten minutes, while those in Table II where 2 c.c. of peroxide were used, faded within three minutes. When a great excess of hydrogen peroxide was added no color developed but a white precipitate formed. When the color fades it is not destroyed but is transformed into a cherry red color which is quite stable.

A few other oxidizing agents were tried in place of the hydrogen peroxide, such as benzoyl peroxide, barium peroxide, and sodium persulphate, but none of them proved as good as the hydrogen peroxide.

By using the proportions of the reagents described in the method, a good color is obtained in dilutions of 1 to 500,000.

Meat extracts, peptone, pepsin, trypsin, and amylase gave no color with the reagents used. Iron salts and iodides do interfere with the test. With iron salts a deep green color is obtained. With iodides a black green precipitate is obtained, and the solution is colored a deep yellow.

TABLE I

TUBE	BLOOD (1 : 10,000)	BENZIDINE	H_2O_2 (3 PER CENT)	*COLOR
1	5 c.c.	0.1 c.c.	0.5 c.c.	2
2	"	0.2 "	"	3
3	"	0.3 "	"	4
4	"	0.4 "	"	5
5	"	0.5 "	"	4
6	"	0.7 "	"	3
7	"	1.0 "	"	2
8	"	2.0 "	"	1
9	"	5.0 "	"	?

TABLE II

TUBE	BLOOD (1 : 10,000)	BENZIDINE	H_2O_2 (3 PER CENT)	*COLOR
1	5 c.c.	0.1 c.c.	2 c.c.	1
2	"	0.2 "	"	2
3	"	0.3 "	"	3
4	"	0.4 "	"	4
5	"	0.5 "	"	5
6	"	0.7 "	"	8
7	"	1.0 "	"	10
8	"	2.0 "	"	15
9	"	5.0 "	"	30

*The intensity of the color is expressed numerically. Number 1 expresses the slightest amount of color observed. The figures are approximate.

In the determination of blood in feces, the tube must be cooled before the reagents are added as otherwise the blue color will go over into the cherry red color immediately.

It is very important to add the hydrogen peroxide last or no color will be produced.

The test was applied to a large number of urines and feces and proved very efficacious. No positive tests were given when no blood was present, as determined by other methods.

SUMMARY

1. A simple method is described for detecting blood in urine and in feces by the use of benzidine dihydrochloride.

2. A simple method is described for the preparation of pure benzidine dihydrochloride from commercial crude benzidine base.

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A SIMPLE METHOD FOR STAINING SPIROCHETES*

By Emil Weiss, M.D., Chicago, Ill.

THE review of the literature shows a considerable number of methods recommended for the staining of the spirochetes. Only few of them are actually in general use due to the fact that most of these methods do not fulfill the fundamental requirements for staining procedures, which are: (1) The applied stain must be effective; (2) the stain must keep well; (3) the procedure must be as simple as possible. The present methods which are used for the demonstration of spirochetes can be classified in four groups: (1) Stains otherwise used for the demonstration of blood cells; (2) methods resembling those used for the staining of flagella; (3) methods with negative pictures (background stained, spirochetes unstained); (4) vital staining.

In using blood stains the spirochetes take the eosin. The affinity of spirochetes for acid dyes is of considerable importance for the interpretation of their biologic status. Bacteria preferably take basic dyes, while the animalic cells show more affinity for the acid dyes; the nuclei of animalic cells take basic dyes. The staining reactions of spirochetes with blood stains indicate a closer relationship to the animal kingdom than to the plant kingdom. The different blood stains demonstrate distinctly the morphology of spirochetes in relation to tissue elements, particularly blood cells. The procedures which resemble the flagella methods use first a mordant, then a basic dye or a metallic salt or a combination of both. The use of metallic salts has one great disadvantage that the formation of sediment cannot be avoided and may cause in some instances doubt concerning the interpretation. The negative methods are undoubtedly inferior to the above methods and may easily cause oversight of the possibly present spirochetes.

The author thought it worth while to compare the different flagella methods as to their usefulness for the demonstration of spirochetes. He found that most of the flagella methods are applicable for the demonstration of spiro-

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chetes. The chief difficulty lies in the fact that the various mordants used by the flagella methods do not keep, and must be renewed at short intervals or filtered before use. Another difficulty lies in the complicated technic of some flagella methods, which require considerable experience to obtain satisfactory results. The author tried to meet these and some other objections in a recent paper on staining of flagella.[3] Further studies also showed that this method is equally useful for the staining of spirochetes.

THE PRINCIPLES OF THE METHOD

Preparing of Films.—The fresh material is carefully placed on a slide and diluted in a drop of 5 per cent glacial acetic acid. The slide is inverted and placed over a hollow ground slide in a manner as usually practiced for the preparation of a hanging drop. A too fast drying of the drop can be avoided by the use of vaseline. The slides with the drop are placed in the incubator for fifteen minutes. The vaseline is then removed with xylol and alcohol. The drop is carefully spread over the slide and dried in air. For routine purposes the material is immediately smeared, allowed to dry and then the mordant is applied. If desired the filming of spirochetes can be preceded by vital staining (Mandelbaum, Meirowsky[1]). The same drop can be utilized for the subsequent application of our method.

The Mordant.—A comparative study of various mordants shows that tannic acid is the most important constituent. The other chemicals are of secondary importance. The deterioration of the majority of mordants is caused by the fact that tannic acid does not keep well in watery solutions. The great solubility of tannic acid in alcohol was utilized to develop a stable mordant. One hundred grams of tannic acid were dissolved in 100 c.c. of 95 per cent alcohol (Sol. A) and before use mixed with two parts of undiluted formalin containing 7.5 per cent glacial acetic acid (Sol. B). This mixture contains about 20 per cent tannic acid. Both solutions are stable and do not require repeated filtering. This mordant resembles somewhat Fontana's mordant, which is not stable. In Fontana's method the fixative and the mordant are used separately. In our method the fixative and the mordant are contained in stable solutions and their application occurs simultaneously. The essential constituents (tannic acid, glacial acetic acid, and formalin) are contained in our mordant in a concentration 4 to 5 times higher than in Fontana's method.

The Use of the Mordant.—The air-dried smears or films are covered with the mordant and steamed slowly for two to five minutes. The removal of the excess of the mordant is most satisfactorily obtained with running warm water.

The Use of Basic and Acid Dyes.—The bacterial flagella and spirochetes show similarity in staining reactions with basic dyes. The bacterial bodies take the basic dyes more heavily than the bacterial flagella or spirochetes. This difficulty can be partially overcome by using saturated solutions of basic dyes, by longer staining, or by application of heat while staining (steaming). Instead of basic anilin dyes metallic salts can be successfully used. The affinity of flagella for acid dyes has been demonstrated in a previous paper.[3] An intensive staining of the spirochetes can be accomplished by staining first with a saturated solution of a basic dye, and then with an acid dye of the same color

(crystal or gentian violet and acid violet, basic and acid fuchsin, brilliant green and acid green, methylene blue and acid blue, etc.).

Contrastive Staining With Basic and Acid Dyes.—This method also devises a procedure for a contrastive staining of flagella by the use of basic and acid dyes. This procedure can also be successfully applied for a contrastive staining of spirochetes. In this instance the bacterial bodies and the nuclei of tissue cells take the basic dyes; the spirochetes, the bacterial flagella, the bodies of degenerated bacteria and the cytoplasm of tissue cells take the acid dyes. Such a procedure is very effective in instances where the smears contain besides the spirochetes a variety of bacteria and tissue elements (for instance, Vincent's angina). The following combinations of contrasting basic and acid dyes are recommended for use: saturated aqueous or alcoholic solutions of crystal or gentian violet and acid green, safranine or fuchsin with acid green, brilliant green with acid violet or acid fuchsin.

Staining Procedure.—The slides which were thoroughly washed with water after application of the mordant, are covered for two to five minutes with the saturated aqueous or alcoholic solution of the respective basic dye, washed off with warm or cold water and then covered for eight to ten minutes with the saturated solution of the acid dye which is dissolved in 30 per cent alcohol. The slides are then washed with water and allowed to dry in the air without application of heat.

DISCUSSION

A method has been described for the staining of spirochetes, which was already found useful for the demonstration of flagella. The spirochetes appear as large as with any of the previous methods, which use mordants. Several dyes can be used with the same results. By the use of basic and acid dyes a contrastive staining between spirochetes and bacteria can be obtained. The special advantages of this method are: (1) The simplicity of the technic; (2) the mordant keeps indefinitely; (3) no sediment is formed; (4) choice between several dyes, and (5) contrastive staining.

SUMMARY

A new method for staining of spirochetes has been devised with the following procedure: The material supposedly containing spirochetes is placed on a slide in a drop of 5 per cent glacial acetic acid. The slide is inverted over a hollow ground slide and placed in the incubator for fifteen minutes. The drop is then spread and allowed to dry in air. The slide is covered with the mordant and steamed for two to five minutes. The slide is then washed with warm water and covered with a saturated solution of one of the recommended basic dyes for two to five minutes; the slide is then washed with water and dried in the air. If a contrastive staining is desired, the slide is then covered with a 10 per cent solution of a contrasting acid dye in 30 per cent alcohol for eight to ten minutes, washed with water and dried.

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AN AUTOMATIC MICROTOME KNIFE SHARPENER AND METHODS FOR GRINDING AND HONING THE KNIFE SATISFACTORILY*

By John I. Fanz, M.D., Philadelphia, Pa.

HAVING run the gamut of razor blade holding devices, having tried all types of hones (including the better grade Belgians), I resurrected the glass plate grinding method of Chas. S. Minot. This produced results far superior to those obtained with the stone hone, but the time required to condition the knife extended over a period of many hours, especially when the knife presented the coarser variety of nicks. To move the attrition glass plate by motor at a suitable speed was the first step in the evolution of the sharpener. To select proper abrasives and to develop methods for their use was the second and most annoying search to make the automatic sharpener a success.

A full description of the instrument would be too lengthy for the present article. Figs. 1 and 2, however, will give a fair idea of the instrument. The 18-inch ground glass disc is rotated at a speed of 42 revolutions per minute, toward the knife edge in all processes of grinding, honing and polishing. Stropping is accomplished on a paper disc covering the glass plate, the movement during this operation being away from the edge. This is accomplished by swinging the knife holder across the disc into an opposite radius. The oblique draw of hand honing is simulated by a reciprocating device, moving the knife inward and outward in the radius of the moving disc 30 times per minute.

MATERIALS USED IN GRINDING, HONING AND POLISHING

1. *Soap Solution.*—This is used in all steps of sharpening, with the exception of stropping. A level teaspoonful of pure powdered castile soap is added to a pint of boiling water in a one liter Erlenmeyer flask, and the mixture shaken thoroughly until the soap is completely dissolved. After cooling under a faucet, the solution is ready for use. All dust particles in flask or drip can must be removed by previous rinsing. The soap must be free of dust and impurities which might scratch.

2. *Emery Flour.*—This abrasive is used for truing the knife edge and grinding out nicks. Only the best Turkish emery flour, FFF, is suitable. It was found that levigation, just previous to use, gave best results in that larger particles were avoided. About ¼ teaspoonful of emery is added to a 20 c.c. test tube filled three-fourths with water. After thorough shaking the tube is brought to rest for a few seconds to allow coarser particles to fall to bottom. The upper ⅞ of the suspension is poured quickly on the revolving disc for grinding. It is better to discard the sediment.

3. *White Rouge.*—This abrasive is a chemically prepared silicon dioxide used by lens makers for polishing optical glass, but becomes a quick abrasive

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for steel. A suspension is prepared, using ¼ to ½ teaspoonful to 15 c.c. of water, shaken thoroughly in a 20 c.c. test tube. The suspension is creamy in consistency and color, and is directly poured on the revolving disc without levigation, in the process of coarse honing.

4. *Diamantine Powder.*—Swiss diamantine powder No. 1 is used to finish honing and to polish the honed surfaces. Diamantine is a crystallized preparation of boron. This powder must be used quite sparingly. An amount heaped over a ¼ inch square surface (about 1 to 2 grains Troy) added to 15 c.c. of water in a 20 c.c. test tube and shaken thoroughly is sufficient for one application in fine honing. A smaller amount suffices for the first step

Fig. 1.—Automatic sharpener. Knife on attrition disk in position for grinding, honing and polishing.

in polishing. Final polishing is accomplished on the clean surfaced attrition disc, using nothing but the soap solution (100 to 150 drops per minute).

5. *Rouge.*—Finely powdered polishing rouge (red iron oxide) is used for stropping. It should never be placed on the attrition disc, but is dusted in small quantity on the paper stropping discs.

INSPECTION OF THE KNIFE TO DETERMINE PROCEDURES TO BE FOLLOWED

The edge should be examined throughout its entire length with a microscope or with a high power magnifier. The ⅔ objective with 10x ocular is the best combination (using reflected light). Great nicks are those which involve all or most of taper length. These necessitate longer grinding with emery suspension, from three-fourths to two hours, depending on the hardness of

the steel. Usually the nicks encountered in a knife which has not been abused are smaller, involving but a portion of the taper surface. These usually yield in from twenty minutes to one hour. Slight nicks and burred edges involve but very little of the taper surface, and are visible to the naked eye only on careful inspection when good illumination is provided. Under the microscope these are readily discernible as serrations and bendings of the edge. Diamantine or white rouge honing without grinding will usually remove these slight imperfections, which, however, are so prohibitive to good sectioning. Knives, which present a belly at middle from repeated stone sharpenings, require

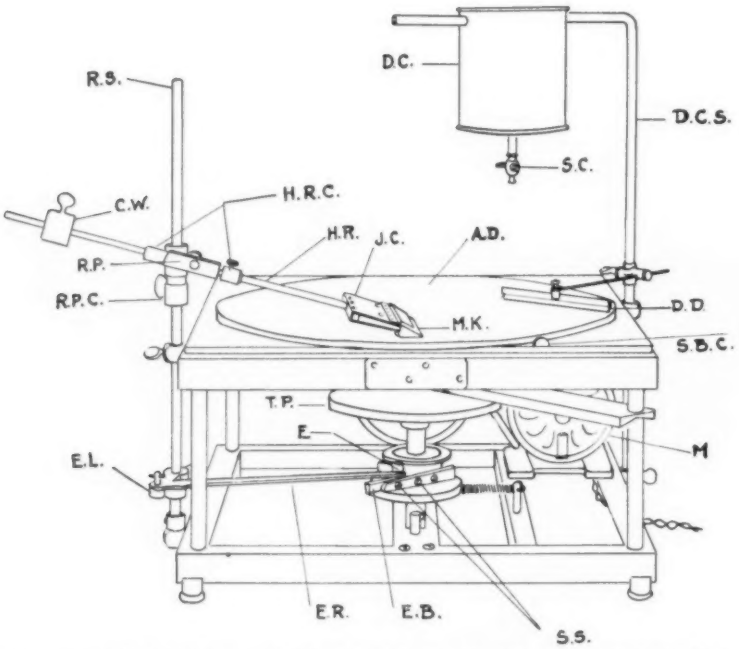


Fig. 2.—Automatic sharpener. Parts labelled for identification. Note knife on attrition disc is now in position for stropping. Paper disc not shown. (Drip deflector *D.D.* is not used in process of stropping.)

D.C., drip can;
S.C., stopcock;
D.C.S., drip can support;
D.D., drip deflector;
R.S., reciprocating shaft;
C.W., counterweight;
R.P., rest piece on reciprocating shaft;
R.P.C., rest piece collar;
H.R.C., handle rod collars;
J.C., jaw clamp of knife holder;
H.R., handle rod of knife clamp;
M.K., microtome knife;
A.D., attrition disc;
S.B.C., spring brass clip, of which three grip the disc;
T.P., transmission speed reducing pulleys;
E., eccentric;
E.R., eccentric rod;
E.B., eccentric block;
S.S., set screws, to fasten eccentric block in any position;
E.L., eccentric lever on reciprocating shaft;
M., motor.

periods of emery grinding from two to three hours or more, dependent on depth of belly and hardness of the steel. In these bellied knives the disc must cut down each end of the knife edge before sharpening at center is possible.

EMERY GRINDING TECHNIC

By means of a carborundum pencil one side of the knife is arbitrarily marked "T" for top, and henceforth must be placed always in the holder to correspond with its top side (marked "T"). Center the knife in the holder. Screw down the two binding screws of clamp so that the knife is held firmly.

Next, wash disc thoroughly under faucet with running water to remove all dust particles, and place same on the carrier head. Lay the knife holder handle in the rest on the reciprocating shaft. Adjust the holder so that edge of knife lies in the near radius of glass disc. With holder in this position, tighten thumb screws on each of the two collars of handle, one collar being set below, the other above the rest piece. (See H. R. C., Fig. 2.) See that both collars are in contact with their respective end of rest piece (R. P.). To govern the length of tapering surfaces to be ground the rest piece is raised or lowered on the reciprocating shaft. By tightening the wing screw of rest piece its position is maintained. To facilitate this adjustment a collar below the rest piece is furnished (R. P. C.) and also provided with a wing screw. It is obvious that by elevating rest piece and collar, one will grind a short taper with a more obtuse edge angle. By lowering rest piece and collar the tapering surfaces forming the edge become longer, the edge angle becoming more acute. For best sectioning results the tapering surfaces should be about 0.5 mm. long; in fact our best knives on measuring with an eyepiece micrometer showed tapers of 0.55 to 0.57 mm. long.

Fig. 3.—Edge of microtome knife after use in sectioning prostatic tissue containing calcific concretions. Not fractured and serrated edge.*

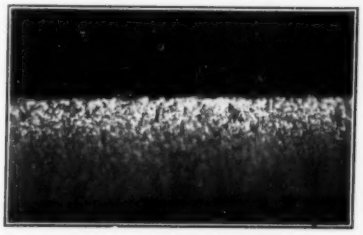


Fig. 4.—Same knife edge after coarser nicks have been removed by grinding 30 minutes with emery flour FFF.

Having adjusted the knife holder for radius position and taper length, the reciprocating movement must be regulated so that one end of the knife reaches the disc edge in the peripheral excursion, the other end almost arriving at disc center on the centripetal excursion or swing. This adjustment is made by shifting the eccentric block and then fastening same by means of two set screws (see S.S., Fig. 2). Before turning on power place at least a pint of soap solution in the drip can and flood the disc. The power is turned on by means of a switch conveniently placed on the power cord. The disc rotates contra-clockwise, moving *toward* the knife edge, which lies always in the near radius for grinding, honing, and polishing. Finally, set the drip deflector (see D.D., Figs. 1 and 2) in light contact with the disc wet with soap solution, in such position as to conserve abrasive and soap solution, and quickly pour on the emery suspension. The knife is turned in the rest piece without stopping the mechanism, by depressing and rotating the handle rod. Two or three minutes is allowed each side before turning the knife over. The soap solution drip is regulated by stopcock under the drip can (see S.C., Fig. 2). From 100 to 150 drops per minute suffice to keep disc from drying. The abrasive suspension is replenished as required. It is a good policy to remove

*Figs 3-8.—Photomicrographs of all microtome knife edges made with 4 mm. objective and 8x ocular, reproduced without retouching.

knife holder containing the knife and to wash and examine the knife every fifteen minutes. The knife should never be removed from jaw clamp until sharpening is fully completed in the stage of polishing or stropping. The grinding must continue until the worst nicks have been completely removed. The fine uniform serrations visible on microscopic examination will remain and further emery grinding will not improve the situation, but in the next step of honing the serrations (provided they are uniform) disappear to give place to the much desired straight line edge (see Fig. 4). The important precautions to be taken in emery grinding are:

1. See that all large nicks are removed.
2. That belly of the knife is trued.

Fig. 5.—Same knife showing effect of grinding with white rouge after the grinding described in Fig. 2. Note straight line edge and long bevel, i.e., double the width of that usually found in a new knife such as shown in Fig. 8. Surfaces converging to edge have not yet been polished.

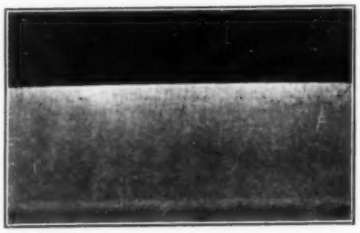


Fig. 6.—Same knife showing edge after polishing on the glass disc of automatic machine with diamantine. Note high polish and long bevel affording unusual strength to cutting edge.

Fig. 7.—Knife edge similar to that shown in Fig. 1 after hand honing and hand stropping by a trained hand. Note wavy edge which, while lacking coarse serrations, is worthless for sectioning tissue.

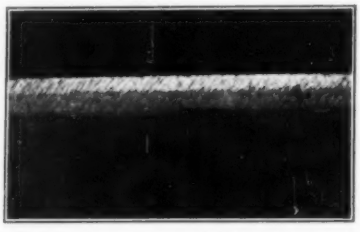


Fig. 8.—Edge of new knife as ground and hand honed by instrument maker. Note narrow, blunt, double bevel and coarse crevices terminating in a serrated edge.

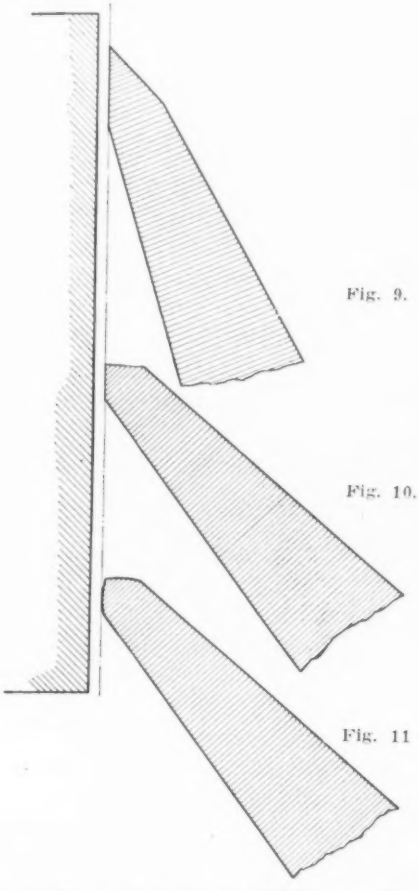
3. That the tapered surfaces converging to terminate in the edge are at least 0.5 mm. long.

4. That the grinding is uniform over the entire length of the knife, i.e., width of taper is the same throughout.

HONING

After the process of grinding has been accomplished, the knife (still in its holder) is thoroughly flushed under a faucet, to rid it and the jaw clamp of all emery particles. The glass disc must also be removed and thoroughly flushed and wiped with a soft cotton towel. The disc is then returned to the carrier head (same side always up). Return the knife holder to its rest, without changing position of same. Flood disc again with soap solution and turn on the power. Readjust the drip deflector and add white rouge suspension

from the test tube, prepared according to directions found in section on "Materials." Five to ten mils, are deposited on disc each time, the disc having been previously wet with soap solution. The knife must be turned every two minutes. As honing continues, the white rouge gets darker due to steel particles ground from the knife. It is well to flush the plate and knife with water every ten minutes, to rid the disc of steel particles which mar the finish. If this should not suffice, a cotton towel wet with ether will entirely remove the stains of steel glaze. The soap solution must be continued to prevent drying of the disc. Examine the knife edge every fifteen minutes with either microscope or magnifier.



SCHEMATIC CROSS-SECTIONS OF UPPER PORTION OF MICROTOME KNIFE TO SHOW LENGTH OF TAPER, INCLINATION OF KNIFE TO BLOCK, MULTIPLE BEVELING, ETC.

Fig. 9.—Knife sharpened on new apparatus. Note acuteness of angle at cutting edge. Compare with Fig. 10. Knife so sharpened permits of its use at a more acute inclination to block surface than knife in Fig. 10.

Fig. 10.—Knife well sharpened by instrument maker. Note angle at cutting edge (more obtuse), making a wide inclination of knife to block surface necessary. This wide angle of inclination tends to make the knife scrape rather than cut.

Fig. 11.—Knife apparently well sharpened, judged by gross visible appearance, but not satisfactory for sectioning. Angle of cutting edge the same as in Fig. 10, excepting microscopic rounding of the two planes forming the cutting edge. This knife is without nicks and considered sharp by usual criteria. The rounded edge makes necessary a still wider inclination of the knife to block surface. If the same angle as in Fig. 10 is retained, the edge of the knife retreats from cutting position. Knives so sharpened skip sections. The rounded edge is usually the result of excessive stropping or careless hand honing.

POLISHING

This step should never be undertaken until the edge, studied microscopically, presents but very fine uniform irregularities which do not improve with further honing (see Fig. 5).

Thoroughly wash the disc under faucet. If disc does not clean well, apply ether on a cotton towel to remove all trace of steel and carbon smudge. Wash the knife and jaw clamp in a similar manner, without removing the knife from clamp. Replace both disc and holder in machine. Flood disc with soap solution, adjust deflector and start motor. Never change the grinding angle of the knife after proper taper has been secured. When disc is running and is uniformly wet, add 5 to 10 mils of diamantine suspension. Polish until taper surfaces present no scratches of note, and until edge is a perfect straight line without appreciable serrations (see Fig. 6). It may be necessary to wash disc as often as every ten minutes to rid its surface of coarser particles and steel which continue to scratch the polished surfaces. The knife should be turned every one or two minutes during polishing and should be rinsed frequently by carrying holder to a faucet and flushing, for the same reason that disc is flushed. It is well to add less and less dosage of diamantine suspension as polishing with this medium continues. Final polishing is accomplished by flushing disc and knife, and allowing knife to remain on running disc kept wet with soap solution alone. Set the drip cock to deliver 150 drops per minute. Take off drip deflector and turn knife every one or two minutes. Examine every ten minutes. The ground surface of the glass disc without abrasive gives a mirror-like surface on the tapers provided grit abrasive and dust particles are eliminated; twenty to thirty minutes usually suffice.

STROPPING

This step concludes the process of sharpening and may improve knives which have been used in sectioning and which lack nicks, or merely present a bent edge. The technic can be outlined as follows:

1. Clean and dry entire apparatus, including knife and holder (without removing knife).
2. Apply a paper stropping disc to glass disc, fastening same by means of the three lugs, one under each spring clip of each carrier head arm.
3. By means of a large soft brush apply sparingly, by dusting, a portion of fine polishing rouge (red oxide of iron).
4. Loosen the wing screw of rest piece and swing the knife across disc to the opposite (far) radius.
5. Now tighten the wing screw securely (do not raise or lower collar below rest piece).
6. Turn on motor.

It will be observed that in stropping, the direction of movement of disc is *away* from the knife edge, instead of toward same, as was the case in grinding, honing, and polishing. The reciprocating motion remains the same. Turn the knife over every minute. Ten minutes of stropping is sufficient.

In concluding, the author wishes to express his appreciation and thanks to his associate, Dr. Edwin S. Gault, for his help, suggestions and cooperation in developing the sharpener.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

PRECIPITIN SERA: Preparation of, with Heated Antigens, Volda, A. D. and Del Carpio, I. Bull. Soc. Ital. Biol. Sper. 3: 935, 1928.

Human blood serum is diluted 1:10 with distilled water and 5 gm. of ammonium sulphate added per 100 c.c. Then add 5 to 6 drops of acetic acid. The mixture is heated in the water-bath until the protein is precipitated, filtered on paper, and the precipitate dried in the incubator. The dried protein is preserved under toluene until ready for use; it is then freed from toluene and emulsified in a mortar with warm physiologic salt solution. The rabbits are then given only 1 intraperitoneal injection of the emulsion containing 1 to 1½ gm. of protein. After seven days, the animals are bled and the serum is tested. The antibodies can be demonstrated in a titer as high as 1:1000. Later the titer then increases and may reach a value of 1:100,000. The antibodies disappear twenty-five to thirty days after the injection. The high titer serum is well adapted for identifying human blood serum or blood spots.

TISSUE: Use of Mixed Dyes in Histologic Technic, Houcke, E. Rev. hyg. et med. prev., Par. 51: 154, 1929.

Solution I. Toluidin blue-orange G.:

Toluidin blue, 1%	10 c.c.
Orange G., 1%	5 c.c.

Add distilled water to make 100 c.c. and allow to sediment for twenty-four hours. The precipitate is collected by decantation. After drying, it is dissolved in 10 c.c. of absolute methyl alcohol. This alcoholic solution contains an excess of the dye.

Solution II. Thionin-eosin:

Saturated aqueous solution of thionin	100 c.c.
Eosin 1%	17 c.c.

The precipitate is treated as above and dissolved in the proportion of one part in 200 of absolute methyl alcohol.

Solution III. Methylene blue-eosin:

Methylene blue, 1%	10 c.c.
Eosin 1%	11 c.c.

Add water to make 100 c.c. Treat the precipitate as above and dissolve 0.10 in 20 c.c. of absolute methyl alcohol.

Solution IV. Azur II-eosin:

To 40 c.c. of a solution of azur eosin 0.04 in 50, add 48 drops of a 1 per cent solution of eosin. Allow the precipitate to sediment. The precipitate is collected upon a filter paper after having decanted the excess of fluid. After drying, the paper is cut in strips which are placed in a flask with 10 c.c. of absolute methyl alcohol. The precipitate dissolves in several days and then the fragments of filter paper can be removed.

Solution V. Toluidin blue-eosin:

Toluidin blue, 1%	10 c.c.
Eosin, 1%	8 c.c.

Add water to make 100 c.c. and treat the precipitate as above, after which 0.10 c.c. of the precipitate are dissolved in 20 c.c. of absolute methyl alcohol.

All of the precipitates obtained in this manner are not equally insoluble in water. The methylene blue-eosin precipitate may be washed 3 or 4 times with decomposition and it is preferable to treat it in this manner, but the precipitates obtained with the other dyes dissociate in water if the lavage is prolonged. These precipitates should not be washed more than once.

The staining is done in dishes from which all traces of acid or alkali have been removed by rinsing with distilled water. To 10 c.c. of distilled water there are added 25 drops of the toluidin-orange or 5 to 7 drops of the other solutions. Two or three drops of a 1 to 1,000 solution of acetic acid are then added. The section is stained for twenty-four hours. After the excess water has been removed the section is washed with alcohol rapidly three times and then placed in xylol and mounted in balsam.

The success of the staining depends upon the degree of acidity of the bath, one drop of the diluted acid more or less will modify the result. If the bath is too acid the eosin and orange alone will stain while if it is too alkaline only the blue will stain. The number of drops of the diluted acetic acid can be obtained for each preparation after a few trials. The stain should never be filtered as filtration removes its activity, the greater part of the dyes being absorbed in the filtering.

This method of staining gives particularly good results for the study of lymphoid and splenic tissue fixed in Zenker's solution, and it is also successful for sections fixed by other methods. The preparation is never destroyed; if staining does not occur in twenty-four hours, it suffices to wash it with alcohol and distilled water and then the stain may be used again after changing the acidity.

BACTERIOPHAGE: A Method for the Study of Bacteriophage Multiplication in Broth, Burnet, F. M. Brit. J. Exper. Path. **10**: No. 2, 109, 1929.

A freshly made filtrate from a culture lysed overnight is titrated by plating successive dilutions (usually tenfold) with the sensitive strain on agar. The plates are incubated at 37° C. until the plaques are clearly visible (five to six hours usually). From the plaque count the volume of the filtrate containing one phage particle is calculated, and a suitable amount added to 10 c.c. of a growing broth culture (four hours) of the sensitive organism to give one particle for each 0.03 c.c. of the culture. After thorough mixing volumes of 0.02 c.c. are removed in capillary pipettes previously calibrated. The pipettes are sealed at the capillary ends and placed in a water-bath at 37° C. At the same time an effort is made to maintain the tube containing the mixture of phage and culture at about 37° during manipulations. As soon as the required number of pipettes have been filled, 40 or 60 in most experiments, 10 lots of 0.02 c.c. are gently blown out on to an agar surface, spread over a reasonable area by tilting the plates, and dried off as quickly as possible in the incubator. At suitable intervals batches of ten sealed capillaries are opened and their contents spread similarly.

TUBERCULIN REACTION: Lovett, B. R. Am. J. Dis. Child. **37**: No. 5, 918, 1929.

Attention is called to the value of the simple percutaneous method described below:

The skin over the sternum is rubbed with ether until hyperemia occurs. A piece of the concentrated old tuberculin, the size of a pinhead, is vigorously rubbed in with the finger tips. The result is read at the end of forty-eight hours. Usually the reaction appears within twenty-four hours, but occasionally it is delayed for three or four days, thus corresponding to the torpid reaction described by Pirquet.

Three grades of positive reactions may be described. The weakest consists of several small scattered papules at the site of inunction, with scarcely any reddening. A moderate reaction is shown by a larger group of papules, with definite reddening. In the strong reaction a marked folliculitis appears, with minute vesicles or even pustules and intense inflam-

mation. There may be some itching. The milder reactions persist for several days; the stronger may be visible for a few weeks. Possible but rare sources of error are diffuse reddening or punctate reddening of the pores without folliculitis as may occur in a sensitive skin. For a positive test there must be definite papules.

ENDAMEBA GINGIVALIS: Cultivation of, Kofoid, C. A. and Johnstone, H. G. Am. J. Pub. Health **19**: No. 5, 549, 1929.

SALT SOLUTION

Sodium chloride	360 gm.
Calcium chloride	8 gm.
Potassium chloride	16.8 gm.
Water	2600 c.c.

Sterilize in autoclave for thirty minutes at 15 pounds pressure.

SODIUM BICARBONATE SOLUTION

Sodium bicarbonate	8 gm.
Water	100 c.c.

This solution must be prepared carefully to prevent absorption of carbon dioxide from the air, and it must not be heated or shaken vigorously. The solution is filtered through a fine Berkefeld candle.

SUGAR SOLUTION

Dextrose	25.1 gm.
Water	500 c.c.

The sugar solution is sterilized in the Arnold for twenty minutes daily on three successive days.

The salt solution is put up in 110 c.c. amounts, the bicarbonate in 5 c.c. amounts and the sugar solution in 10 c.c. amounts. The flasks of salt solution and the tubes of dextrose are capped with paper while the tubes of sodium bicarbonate are corked and covered with paraffine.

To make 2 liters of Locke's solution take a flask of salt solution (110 c.c.), a tube of sodium bicarbonate solution (5 c.c.), a tube of dextrose solution (10 c.c.), and add to 1,885 c.c. of sterile distilled water. The above solutions are made every three months and can be kept indefinitely. Two liters of Locke's solution can thus be prepared in about five minutes, which is of great advantage when needed in a short time or in cases of emergency.

Locke's albumen solution: The white of one egg is carefully cracked into a sterile liter flask containing glass beads, shaken vigorously until frothy; then 500 c.c. of Locke's solution and 20 c.c. of N/20 HCl are added. This quantity of acid proved to be about the right amount to add to a liter of fluid in order to give a P_H 7.4 to 7.6. The remaining 500 c.c. of Locke's solution are added and the whole mixture shaken thoroughly and filtered through a Berkefeld candle. This sterile medium is added aseptically in 6 to 10 c.c. amounts to the egg slants.

It is possible to make the Locke's egg-albumen medium without filtering, but it has been the experience that the filtered medium gives by far the most satisfactory results. The unfiltered medium is prepared exactly as the filtered with the exception that 2 egg whites instead of one to a liter of Locke's solution are used. The eggs must be sterilized in 90 per cent alcohol before cracking and aseptic precautions observed to avoid contamination. An uninoculated control tube should be run at the time of each transplant.

The medium is warmed to 37° C. previous to inoculation with either fresh or culture material. Subgingival exudate or pus is removed by sterile sharpened applicator sticks or

sterile dental scalers, and introduced into the culture tubes. The tubes should be placed immediately in the incubator at 37° C. and at no time should they be subjected to prolonged chilling.

Cultural transplants are made by removing a small amount of the material which collects in the angle formed by the egg slant and the side of the tube with a sterile Pasteur pipette and inoculating this substance into fresh medium warmed to 37° C. Transplants are made every forty-eight hours.

BLOOD: The Volume and Hemoglobin Content of the Red Blood Corpuscles, Wintrobe, M. M. Am. J. Med. Sc. 177: No. 4, 513, 1929.

The following formulae for the calculation of the "corpuscular volume" are presented:

$$\frac{\text{Red cell volume in c.c. per 1000 c.c. of blood}}{\text{Red cell count in millions (and fractions) per c.mm.}} = \text{corpuscular volume in cubic microns (10 to 12 c.c.)}$$

$$\frac{\text{Hemoglobin (gm. per 100 c.c.)}}{\text{Red cell count (millions per c.mm.)}} = \text{"corpuscular hemoglobin"}$$

The actual proportion of the substance of each red cell taken up by hemoglobin may be calculated by dividing the corpuscular hemoglobin by the corpuscular volume; or, directly, by dividing the hemoglobin content of a given sample of blood, expressed in grams per 100 c.c., by the total cell volume expressed in c.c. per 100 c.c.

The example illustrates the calculations:

A sample of blood is found to contain 5.85 million red cells per c.mm., 15.87 gm. of hemoglobin per 100 c.c. (or 158.7 gm. per 1000 c.c.) and 46.5 c.c. of packed red cells per 100 c.c. of blood (465 c.c. per 1000 c.c.). Then, the average corpuscular volume is $\frac{465}{5.85} = 79.5$ cubic microns (or 79.5×10^{-12} c.c.); the average corpuscular hemoglobin is $\frac{158.7}{5.85} = 27.1 \times 10^{-12}$ gm.; proportion of hemoglobin in the average cell is $\frac{27.1}{79.5} \times 100 = 34.1$ per cent (or $\frac{15.87}{46.5} \times 100 = 34.1$ per cent).

It is obvious that the methods used for the preliminary determinations must be accurate.

GRAM STAIN: Modification of, Zeissig, A. Stain Technol. 4: No. 3, 91, 1929.

1. Apply nuclear stain.
2. Wash.
3. Stain in Hucker's gentian violet two to three minutes (i.e. 1 part saturated alcoholic solution crystal violet to 4 parts 1 per cent aqueous solution ammonium oxalate).
4. Wash in water.
5. Stain in Gram's iodine five minutes.
6. Wash in water.
7. Decolorize in 95 per cent alcohol to which enough tincture of iodine has been added to give a mahogany color.
8. Counterstain.
9. Dehydrate and mount.

PEROXIDASE REACTION: A Modification of the Peroxidase Reaction, Etc., Strumia, M. M. Arch. Path. 5: No. 3, 447, 1928.

The following modification can be used not only for blood smears but also for frozen sections (formalin-fixed).

Two stock solutions are prepared:

Solution A: Sodium nitroprusside, 5 per cent aqueous solution.

Solution B: Benzidine, 2.5 per cent alcoholic solution.

For use, mix 1 c.c. of Solution A and 95 c.c. of 95 per cent alcohol; add 2 c.c. of Solution B and 2 c.c. of fresh solution of hydrogen peroxide.

This solution must be allowed to ripen for an hour or more to give the best results. While this mixture keeps at least as well as the original Goodpasture solution, the stock Solutions A and B keep indefinitely; the mixture can easily be prepared each time it is needed by mixing the stock solutions in proper proportion.

The technic of staining is the same as in the original Goodpasture method. The dried blood smear (which should be fresh) is covered with the solution for one to two minutes. An equal amount of distilled water is then added, and the preparation is stained for from three to five minutes until, under the low power of the microscope, blue granules appear in the polymorphonuclears and large mononuclears. The slide is then washed in running water for about fifteen minutes.

TULAREMIA: A Presumptive Test for, Hull, T. G. Am. J. Pub. Health **19**: 4, 423, 1929.

CULTURE MEDIUM FOR BACTERIUM TULARENSE. BLOOD GLUCOSE CYSTINE AGAR

Fresh beef infusion agar, containing 1.0 per cent peptone, 1.5 per cent agar, and 0.5 per cent sodium chloride, adjusted to a P_H of 7.3 is kept on hand in stock. When needed, there is added to the stock agar 0.5 per cent of cystine and 1.0 per cent glucose, and this is heated in an Arnold steam sterilizer sufficiently long to melt the agar and to sterilize the cystine and glucose, after which it is cooled to 40 to 45° C., when 5 to 8 per cent defibrinated rabbit blood is added. Overheating and loss of the bright red color should be avoided. The medium is then tubed, slanted and incubated to insure sterility.

The addition of the cystine does not change the P_H of the medium, but if cystine hydrochloride is substituted for cystine, a correction may be necessary on account of acidity. Cystine is not very soluble in the beef infusion peptone agar, and for that reason it should be pulverized before being added; even then, visible particles settle in the medium.

Tubes in which the water or condensation has evaporated are preferable. The organism scarcely grows in a liquid medium. Freshly prepared tubes with moist surface and with abundant water of condensation should be allowed to stand with cotton stoppers, in a slanted position, in the incubator at 37° C. for about one week, until almost free of water of condensation. They are then stood upright and plugged with cork stoppers soaked in a very hot mixture of 1 part vaseline and 2 parts paraffin, to prevent further evaporation; then they are stored in the cold room.

Stock cultures are kept in the cold room and transferred every two months. Cultures are stoppered with cotton during two or three days of incubation but with paraffin cork during two months in the cold room at 5 to 10° C., preferably 5° C.

A large loopful of growth is carried over when making transfers. A control tube of plain agar should show no growth.

The blood in the medium conduces to luxuriance of growth and to longevity of life of the culture; 8 per cent is preferable to 5 per cent. Human blood might be more accessible than rabbit blood and might serve equally well.

MAKING THE ANTIGEN FOR AGGLUTINATION TESTS

Blood is omitted from the medium when growing antigens for agglutination or absorption tests. The medium then becomes glucose cystine agar.

Blake bottles containing glucose cystine agar are employed when a large quantity of antigen is desired. Before being inoculated the bottles should dry several days in an inverted position in the incubator, and any water of condensation should be pipetted off before inoculation. Each bottle is inoculated with the entire growth from a blood glucose cystine agar slant suspended in 1 c.c. of physiologic saline solution. This suspension is quickly spread over the surface of the medium in the Blake bottle by rocking the bottle in the hands, all excess of fluid being absorbed by the dry medium.

Incubate the bottles in their normal noninverted positions at 37° C. for three days. Take off the growth of each bottle in 15 c.c. of physiologic saline solution containing 0.2 or 0.3 per cent of formalin (U. S. P. strength, 37 per cent). Thrown down the bacterial mass in the centrifuge, thereby washing the organisms. Pour off the fluid. Take up the bacterial

mass in saline containing 0.2 or 0.3 per cent formalin. This concentrated stock suspension has been found to be entirely reliable for agglutination tests even after storage for two years in the cold room. Portions of the concentrated suspension are diluted at the time of use with saline solution to the desired turbidity, using any method of determining the turbidity, the simplest, cheapest and most practical of which is the silica standard.

THE PRESUMPTIVE TEST

One drop of concentrated antigen is mixed on a glass slide with one drop of serum, either diluted or undiluted. Within thirty to sixty seconds the reaction is usually complete, as easily determined when the slide is tilted back and forth against the light. This method does not give reactions in as high dilutions as the slow test in tubes because the material dries up too soon. Protected from drying by a vaseline ring and cover glass, however, the results are almost identical by the two methods. Dried blood submitted on foil may be moistened with distilled water and tested with very satisfactory results.

LEAD POISONING: The Blood of Workers Exposed to Lead, Mathew, R. Y. Med. J. Australia **16**: No. 1, 208, 1929.

The following methods are preferred by the author for the demonstration of basophilia.

SELLERS' METHOD

To prepare the stain the following formula is used:

Methylene blue (pure medical)	1 gm.
Sodium bicarbonate	6 gm.
Distilled water	200 cm.

The stain is good for six weeks.

To stain film: (1) Fix with methyl alcohol for three minutes; (2) stain for half a minute; (3) wash well in tap water until the film becomes a pale greenish color. Dry with blotting paper.

Appearance of cells showing punctate basophilia is as follows: The red cells are stained a faint greenish color. The basophilic granules show as dark blue dots in the red cells.

GILBERT'S METHOD

To prepare the stain use the following formula:

Methylene blue	2 gm.
Sodium bicarbonate	12 gm.
Distilled water	200 c.c.

To stain the film: (1) fix in methyl alcohol for three minutes; (2) stain for one minute; (3) wash well in distilled water until the color almost fades. Dry with blotting paper.

The appearance of punctate cells is similar to that described for Sellers' method.

B. DIPHTHERIAE: Stain for, Gutstein, M. and Neisser, H. Centralbl. Bakteriol. **108**: 253, 1928.

1. Stain two to five seconds with 1 per cent aqueous Azure II.
2. Wash in water.
3. Differentiate with 3 per cent acetic acid (one to three seconds).

Granules are reddish-*violet;* bodies blue.

or

1. Stain two to three minutes with 1 per cent aqueous Azure II.
2. Wash with water.
3. Wash with aqueous chrysalinine.

Granules are black; body yellow.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*Old Age**

THIS arresting essay is reviewed in the editorial pages of this issue.

Serodiagnosis of Syphilis†

THIS second report from the Health Organization of the League of Nations records and analyzes the results of 944 tests by various methods, the technic of which is presented in full in an appendix to the report.

The methods used during the conference were:

1. Complement-fixation tests of which there were several varieties: (a) That known as Method No. 1 of the British Medical Research Council; (b) The Blasi modification; (c) The method of Calmette and Massol, modified by Debquins; (d) The synoptic method of Jacobsthal; (e) The original Wassermann (carried out by German and Serbian workers), and (f) The method of McIntosh and Fildes (as used by Polish workers).

2. Flocculation Tests: The Kahn reaction; Meinecke's Turbidity Test (M.T.R.); Müller's Clotting Test (M.B.R.); Murata reaction; Sachs-Georgi (Cito- and Lentochol reaction); the Sigma reaction (modified); and the Vernes reaction.

All the tests mentioned are described in detail in the appendix, a section of some interest to all who have followed or been engaged in the evolution of serologic technic.

It seems somewhat surprising to the reviewer to note the emphasis laid upon certain phases of technic in the modification of the Wassermann test, the principles of which have been more or less generally recognized for nearly a decade by American serologists; to note the extent to which the original Wassermann technic still holds sway in Europe with relatively minor modifications—Otto and Blumenthal, for example use as antigen an extract of syphilitic liver—and, in general, the absence of many of the technical refinements based upon the studies of American serologists. It would appear, in fact, that the studies of American serologists in the last decade have been rather generally overlooked or ignored by European investigators.

In a comment which appeared in this Journal (January, 1926) upon the first report of the League of Nations upon serodiagnosis in syphilis, it was said: "It is difficult to avoid the impression that . . . . there was an effort . . . . to show that each of the methods was an excellent method in the hands of its originator or proponent." The second report likewise carries a suggestion of the same impression.

The details of the report are of interest to all engaged in serologic examinations. Kahn, for example, suggests a still further modification in the method of precipitating the globulin from spinal fluids for examination (use of small tubes, 7.5 by 1 cm. and 50 per cent instead of 40 per cent ammonium sulphate solution) and Col. Harrison comments specifically upon the unsuitability of the Kahn test for use with cloudy spinal fluids, while Professor Vernes comments upon the fact that the conference depended upon +, −, and ±

*Old Age. The Major Involution. The Physiology & Pathology of the Aging Process. By Aldred Scott Wathin, PhD., M.D., LL.D., Professor of Pathology and Director of the Pathological Laboratories in the University of Michigan, Ann Arbor. With 29 illustrations. Pages, 200. Hoeber, New York, 1929.

†Report of the Second Laboratory Conference on the Serodiagnosis of Syphilis, League of Nations Health Organization, Geneva, 1928, Cloth, p. 186.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

results. As he said: "It was through sustained clinical observation that we were led nearly twenty years ago to the fundamental conclusion that serology would be condemned to remain a rudimentary science if it could only produce + or − results, while in clinical practice and all other laboratory sciences, *fluctuations* are observed and noted. At the conference, however, in order that our results might be compared with others, we were asked to express them by the symbols +, ±, and −, which we thought we had buried forever when we established our first scale in 1910."

Perhaps in the future, once the question of relative specificity is settled to the satisfaction of the Conference, attention to the vitally important matter of *quantitation* of serologic reports will be studied.

It is to be hoped that from some future conference will come a determination of the best available European method for the conduct of the complement-fixation test, for then it can be compared with the more outstanding American methods to the great interest of all concerned.

The conclusions of the Conference are of decided interest, so much so as to warrant extended quotation:

It is of the opinion that "the best of (the flocculation tests) are equal in value to the best of those which depend upon the fixation of complement."

The Conference "desires, nevertheless, to emphasize the fact that, no less than the complement-fixation tests, these flocculation methods are, despite their apparent simplicity, extremely sensitive to the slighest differences in experimental conditions and subject to so many sources of error, in connection both with the execution of the test and the reading and interpretation of the results, that they must be placed only in the hands of specially trained serologists."

This conclusion is of especial interest in view of the agitation following the introduction of the Kahn test for its *exclusive* use in the serologic study of syphilis.

In this connection, 17 of the 38 workers at the Conference express the opinion that: "(a) As theoretical considerations give reason to expect, some sera react to the Bordet-Wassermann but not to the flocculation tests and vice versa, and that the Bordet-Wassermann and the flocculation tests supplement each other; (b) That strong confirmation of a weak or ± flocculation test is afforded by a positive Bordet-Wassermann test and vice versa, ..." these workers "prefer that one of the methods used should be a Bordet-Wassermann test."

The Conference expresses the opinion that "in order to secure the most reliable information to the clinician, at least two different serodiagnostic methods should be used, to which the paragraph above is an addendum.

"It is a matter for some surprise that the Conference suggests a return to merely plus and minus reports which in view of the many studies, it may be doubted will assist to the fullest extent clinicians . . . . in observing progress under treatment."

Serologists in general will be most heartily in accord with the following: "The Conference desires to record its view that considerable misunderstanding would be avoided and reports on tests of sera would be greatly enhanced in value if clinicians would study closely the diagnostic and therapeutic implications of such reports."

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*The International Medical Annual**

THE fact that medicine is an ever changing art has had within recent years a noteworthy effect upon the character of medical books.

The ponderous, encyclopedic "systems" of yesteryears have given way in no small measure to smaller and more flexible texts which, in turn, are supplemented by the monographic contributions of specialized authorities.

The newer advances, the trials, failures, and successes of new methods and the modern adaptation of older ones appear, however, in the relatively ephemeral journals. Unless one

*The International Medical Annual, 1929. A Year Book of Treatment and Practitioner's Index. Edited by C. F. Coombs, M.D., and A. R. Short, M.D., Cloth, 568 pages, 71 plates and 89 text figures. William Wood and Co., New York.

has access to, and time to read, all, or at least the greater majority of these it will not be long before, to some extent, he will fall behind the times.

To meet just such a situation, even more acute now than it was then, the International Medical Annual was begun forty-seven years ago. The present volume, the work of thirty-one contributors, presents in an eminently readable manner a comprehensive yet succinct summary of the advances made during 1928 in the treatment of disease by all the many means comprised in the generic term "the practice of medicine."

The material thus reviewed, digested, and summarized is presented in a most practical manner as a "Dictionary of Practical Medicine."

Each subject is arranged in alphabetical order and there is, moreover, a very complete index and cross-index so that the particular item desired may be readily found.

Although the work of English authors, the catholic character of the book is evidenced by the cosmopolitan array of the sources from which the reviews have been compiled. Not only the literature of Europe but the literature of the United States is well represented in the survey of the advances of the year.

Interspersed throughout the book are original contributions of practical value. The illustrations are excellent in every way.

For the practitioner at large this is an exceedingly practical and useful volume well deserving of a handy niche in the office where it can be conveniently and frequently consulted.

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EDITORIALS

Medical Mysteries of the American Wilderness

WITHIN the wildernesses of the earth have always lurked many of man's deadliest enemies. While Moses and the Israelites were eking out a hungry and disconsolate existence in the wilderness of Sinai we read[1] that "there went forth a wind from the Lord, and brought quails from the sea, and let *them* fall by the camp, as it were a day's journey on this side, and as it were a day's journey on the other side, round about the camp, and as it were two cubits *high* upon the face of the earth.

"And the people stood up all that day, and all that night, and all the next day, and they gathered the quails: he that gathered least gathered ten homers: and they spread *them* all abroad for themselves round about the camp.

"And while the flesh *was* yet between their teeth, ere it was chewed, the wrath of the Lord was kindled against the people, and the Lord smote the people with a very great plague.

"And he (Moses) called the name of that place Kibroth-hattaavah: because there they buried the people that lusted.

"And the people journeyed from Kibroth-hattaavah unto Hazeroth; and abode at Hazeroth."

While in this instance, as usual, Moses and the Lord operated on a scale of bewildering magnificence, yet in the more modest circumstances of recent times many of the features of this early plague have again manifested themselves. The primitive forests, streams, swamps, prairies and deserts of the early United States constituted an excellent setting for a number of the pestilences which have affected man or beast or sometimes both. While in the beginning profound mystery surrounded all these plagues which had their origin in the "wrath of the Lord" or in some similar etiology, yet one by one most of these mysteries have yielded to the eager, inquiring spirit of the modern epidemiologist and research worker.

Of the early American epidemics perhaps none was more mysterious or stealthy in its approach than was the malady known as "milk-sickness" or the "trembles." Already known to the Indians this disease continued like an hereditary scourge to blight great sections of the territory lying roughly west of the Allegheny mountains, east of the Mississippi River, northward to the Canadian border and southward to Georgia and Alabama. Accounts of the disease were left by Barbee[2] in 1809 and by Telford and Stewart[3] in 1812. But the first comprehensively accurate and complete account of the malady was given by Daniel Drake[4] in 1841. Drake made a series of long and dangerous journeys throughout a large section of the affected region. While he personally saw no cases of the disease, still he collected all the data that he could possibly obtain from the natives and the physicians of the region (several of whom had attended many cases) and he made an extensive study of the botany, the geology, and of the climatic conditions of the country. The disease attacked both animals and human beings, apparently being transmitted to the latter by the drinking of milk from affected cows, or from the eating of meat from animals having the disease. Drake listed eight animals as being subject to the disease, viz., the cow, the horse, the sheep, the hog, and the dog, to which he added as doubtful, the goat, the mule, and the buzzard. The treatment included blood letting, cathartics, opium, counter-irritants, cold affusions, antacids, alcoholic tinctures, and other "diffusible stimuli," drinks, such as a coffee made of scorched oats, and infusion of wheat or weak chicken broth.

Regarding the etiology of the disease, Drake, after making many observations and considering many possible origins finally announced his conclusion that the disease was caused by the Rhus toxicodendron which was eaten by the cattle. That was in 1841. It is interesting to note that Beach,[5] writing in 1887 was able to summarize the accumulated knowledge of his day on the subject by stating that "first there are the theorists who cling to the idea of ingestion, by the herbivora, of some injurious form of vegetation; second, those who attribute it to a deleterious water supply; third, the marsh miasmatic theorists; fourth, those who attribute it to some mineral poison that has an affinity for moisture, and, rising with the dews, settles on and

adheres to vegetation, or, rising with the dews, is taken in by inhalation; fifth, those who believe that its cause is bacterial—that spores, bacteria, or some microscopic fungi or disease germs are taken into the system with either food, the water, or the inspired air."

These suggestions obviously represent the 1887 version of the "wrath of the Lord." And while various writers[6] of the present day have considered the disease to be caused by Eupatorium urticae folium (white snake root), Jordan and Harris,[7] on the other hand, in 1907 found no white snake root in certain regions where the disease prevailed, and they considered it to be due to the Bacillus lactimorbi. And Baldwin[8] writing in 1927 found it necessary to state that "the specific etiology is in doubt." It is worth while to reflect that during a large part of the last century it was often necessary for the settlers in affected regions to give up and desert large tracts of valuable land on account of the disease. In fact, they were still "journeying from Kibroth-hattaavah unto Hazeroth."

Another disease of somewhat similar character and history is the "loco disease," or "locoism." A considerable number of plants have been considered as causes of this disease. Kramer[9] mentions the California loco-weed (Astragalus crotalariae), the Texas or woolly loco-weed (A. mollissimus) and rattle-box (Crotalaria sagittalis). He also states that "Clitoria glycinoides of Brazil, Phaca ochroleucaca of Chile and Oxytropis lambertii of Mexico are poisonous to horses and should probably be included with the loco-weeds."

Following up the investigations of a number of other excellent workers[10] in this field, Couch[11] has recently published an extensive series of very interesting experiments on locoism in which cats have been used as experimental animals. He has worked with an extract of Oxytropis lambertii and has succeeded in isolating from the extract a highly toxic fraction which will produce typical symptoms of the disease in cats.

Although Couch has not succeeded in isolating the loco-poison in a pure state he has obtained it sufficiently free from foreign substances to furnish some definite information concerning its nature. It is very soluble in water and extremely hygroscopic, less soluble in alcohol and methanol, and still less soluble in acetone. It is quite insoluble in ether, chloroform, and the hydrocarbon solvents. It is unaffected by boiling with dilute mineral acids or alkalies, which indicates that it is not of either ether or glucosidal nature. It does not reduce alkaline copper solutions and is not basic. No precipitant has been found for it. It does not affect the ray of polarized light and it is free from barium compounds.

Thus little by little the "wrath of the Lord" is being confined within narrower and narrower limits, and the necessity of fleeing from "Kibroth-hattaavah" to "Hazeroth" grows less and less.

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—*D. E. J.*

The Aging Process

TO MANY the conception of personal immortality is a beautiful idea, to be devoutly prayed for. To most of us the possibility of prolongation of existence on this earth, preferably as a happy existence, offers more promise of an immediate fulfillment. The fountain of youth, whether it be a new supply of drinking water, a commercialized spa, a testicular transplantation, a vasectomy, an endocrine rearrangement, a new wrinkle in calisthenics, Metchnikoff's Bulgarian bacillus, or any of the thousands yet to be proposed, will always seem just around the corner. Even the great Bernard Shaw, basing his ideas on a little biologic knowledge, overlooks the vital fact and, in his "Back to Methuselah," tickles the palate of those hedonists who would stay young long and grow old gracefully. Probably he wrote it with his tongue in his cheek.

We hear much of the prolongation of the average life expectancy. True, in the forty years from 1880 to 1920, twenty years were added to the average duration of human life. This is a wonderful achievement, accomplished principally by the saving of infants and children, and fortunately it has carried with it increased comfort, health, and happiness to those who are living longer.

But it is quite a different matter to talk about lengthening the individual life span beyond its present limitation of ninety to a hundred years. In the period from 1880 to 1920 the average life was longer and there was indeed a progressive increase in the number of persons reaching the ages from eighty to ninety. But above ninety there has been no increase whatsoever in the proportion of survivors.

In the last fifty years more folks have grown older but these older folks have been unable to add still more years to the maximum of a hundred and more years ago.

Of course we would like to live to a ripe old age and more particularly to maintain our health during the living. Thanks to the advances of the medical and allied sciences this goal is slowly being approached. But can we further cheat the grim reaper by widening the life span, say to 125 or 150 years? For the welfare of the race, would it even be a desirable development?

Aldred Scott Warthin thinks not. In a charming volume on old age Dr. Warthin presents his thesis on aging or as he calls it *the major involution.*

Living matter is granted the lease of life as long as it has a function to perform and performs it. The function having been accomplished and completed, deterioration sets in. An unused elastic will lose its "life." A spare tire will rot more rapidly than one on the wheel. Man's function in a biologic sense is to reproduce his kind and then to live long enough to protect his offspring until the latter becomes independent and self-sustaining. After that, biologically, he has no further function to perform. It would be splendid to see the great minds live to an old age, helping direct the progress of evolution and retaining their brilliancy to the end. It is more than pathetic to see the once brilliant mind lose its luster, but continue to exist in a desultory fashion in a body that has lived too long. The vast majority of persons who today reach senility are dependent on others, usually their children, for continued existence. At the present stage of our evolutionary program the prolongation of the total life span would mean but the increasing of the number of senile dependents.

Will the prolongation of health and efficiency into the higher decades that is admittedly occurring today result in an extension of ability, efficiency and independence into what are now the senile years, with a consequent setting back of these latter into still higher decades? Warthin believes not. Indeed, in accordance with his theory of degeneration after completion of function, men in the future should live not quite so long, instead of longer, since nowadays the offspring reaches independence at a somewhat earlier age.

Whatever corroboration the future may or may not bring on this, Dr. Warthin's discussion of those involutionary changes which follow cessation of function is most illuminating.

Involution commences practically synchronously with fertilization. The spermatozoon once having penetrated the ovum, motility is no longer requisite, and the tail of the spermatozoon rapidly involutes. Both the ovum and the spermatozoon suffer a reduction in chromosomes, which means involution. Even in embryonic life involution keeps pace with evolution. The branchial clefts subserve a function in determining the conformation and arrangement of various organs and structures of the head region, and then disappear. The notochord which marks the transition from the invertebrate stage of the embryo to that of the vertebrate, later disappears. The embryonic tail becomes incorporated within the body of the coccyx. Of the several mammary glands which commence to develop, only the two breasts persist. These are only a few of the several embryonic organs which serve some function and then disappear. It is in the placenta especially that we first see involutionary processes which are later comparable to the involution of man himself. At birth the placenta is a senile structure presenting sclerosis and obliteration of arteries, fibrosis of stroma, atrophy and degeneration of the chorionic epithelium, thrombosis, infarction of villi, calcification, etc. "All of the evolutionary tissue changes of old age appear in this important vascular organ at the completion of the period of intrauterine life. In the fulfillment of its function the placenta passes through well-defined stages of evolution, maturity, and senescence, and when its work is over it is discarded as useless, and disappears from the scene of the individual's life.

"In the newborn child, blood vessels which served a function during embryonic life but which, with the onset of respiration and after the severing of the umbilical cord, have no further function to perform, degenerate and become obliterated, very much as occurs in the general circulation in old age."

In the majority, or in all of the vascular involutions of the developmental period, the exciting cause of these premature senescent changes appears to be a *loss of usefulness, a reduced function* and *not overwork*. The characteristics of these early vascular changes are identical in kind with those of the arteriosclerosis of later life. The changes are associated with disuse rather than overuse. They are not wear-and-tear changes nor are they toxic. The thymus, when it has completed its function whatever it may be, atrophies, with disappearance of parenchyma, obliteration and sclerosis of blood vessels, fibrosis and fatty infiltration.

Involutionary changes occur in the ovary after each menstruation, with resulting sclerosis and calcification. At the menopause the ovary as a whole is as distinctly a senile organ as was the placenta at full term. "It is much reduced in size except for cyst formation, irregularly scarred on its surface, and fibroid in consistency. Microscopically it consists chiefly of sclerotic blood vessels and the remains of corpora fibrosa, with here and there a cystic follicle."

These same vascular involutionary processes are so pronounced in the involuting uterus following pregnancy as to be of diagnostic value, where there is a question of preexisting pregnancy.

The essential tissue changes of all of these minor developmental involutions are the same as those that occur in the body as a whole with senility. They consist of parenchymatous atrophy and degeneration with vascular obliteration and sclerosis.

The minor involutions occur in specialized organs and tissues which have completed their function and are destroyed for the good of the individual. The major involution or senescence occurs in all of the organs and tissues because the individual has fulfilled his usefulness and it is time that he make place for others. The minor involutions take place for the good of the individual, the major involutions for the good of the species.

A man who lives too long is apt to hinder evolution rather than help it. It is by continuous creation of new forms of the same life, better adapted to the ever-occurring changes in the environment, that evolution in its broad sense occurs. "The universe, by its very nature demands mortality for the individual if the life of the species is to attain immortality through the ability to cope with the changing environment of the successive ages."

We are here on this earth, primarily not to perpetuate our individual existences but to perpetuate the species. The energy charge known as life is greatest in the original fertilized cell and from this instant there is a gradual but progressive diminution in energy charge up to and through old age. The life of the species is perpetuated by repeated renewals of the energy charge.

The energy charge is expended at a constantly diminishing rate through the period of growth and development until maturity is passed, after which it declines still more rapidly until the machine finally comes to a standstill. This termination of the activities of the protoplasmic energy machine due to loss of its intrinsic energy is normal or biologic death. Growth, that is, cellular activity, is greatest during the first month of intrauterine life. Growth during the second month is relatively greater than that from the period of birth to maturity; in the third month it equals relatively that of the first six years of extrauterine life; and in the fifth month it is as great as in the first year of life. After birth the growth energy is strongest during the first year. During this time height is increased about 50 per cent and weight about 200 per cent.

At maturity the balance between cell growth and cell destruction becomes equalized so that repair is possible without loss of tissue elements and the curve of nutrition and metabolism remains relatively level until the onset of senility, when the energy curve again drops off rather rapidly.

Fortunately for our peace of mind, according to Warthin the psychic function continues to evolve as a rule up to about seventy years, before it too involutes.

Normal biologic death appears to be rare. Disease and other catastrophes of our daily life usually terminate the process sooner. Premature involutionary changes are not infrequent. We might say that in such persons death is still biologic but that their normal life span is for some intrinsic reason shorter than the average.

This raises the question whether the therapeutist may hope to accomplish anything for the individual in the treatment of premature degenerative changes such as arteriosclerosis, myocarditis, nephritis, other than to the extent of protecting the individual by conserving his energy output. Must we admit defeat in the face of premature degenerative changes and inform the patient that he has fulfilled his function and must be prepared to continue on the downward physical path?

Or may we still cling to at least a remnant of the wear-and-tear hypothesis and try to lead the victim out of his predicament by early recognition, removal of infectious foci, prevention of toxemic absorption, avoidance of metabolic overload and the like?

There is no gainsaying that some are predestined from birth and by inheritance to premature senile changes. Will wear and tear intensify this innate predisposition or is it exclusively a matter of functional involution? Dr. Warthin's thesis explains the changes of senescence. It enables us to understand the pathology of premature senescence. But does it explain the cause for premature senility? Is it entirely a matter of heredity or may it be influenced by extrinsic and intrinsic constitutional factors which enter into the picture after birth? Warthin does not answer this question although he does describe premature old age as an intrinsic pathologic cause of death rather than as biologic death. It is a question that none can answer at the present time with certainty but yet one of the utmost interest.

Certainly we would all agree with Dr. Warthin in his views on rejuvenation. Gland transplantation and vasectomy may temporarily stimulate the sexual appetite but it will have no effect on the major involution and, like new wine in old bottles it may unfortunately be sufficiently stimulating to be responsible for fatal catastrophy. Like Warthin we would speak not of rejuvenescence but of reerotization.

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—*W. T. V.*

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THE 1929 WARD BURDICK RESEARCH AWARD OF THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS TO

WALTER MALCOLM SIMPSON, B.S., M.S., M.D., DAYTON, OHIO

THE first Research Committee of the American Society of Clinical Pathologists in 1928 planned a fitting memorial to one of the founders and the first Secretary-Treasurer of the Society by the institution of the Ward Burdick Research Award in the form of a gold medal bearing a profile image of the late Dr. Burdick on one side and the Society seal and name of the recipient on the reverse.

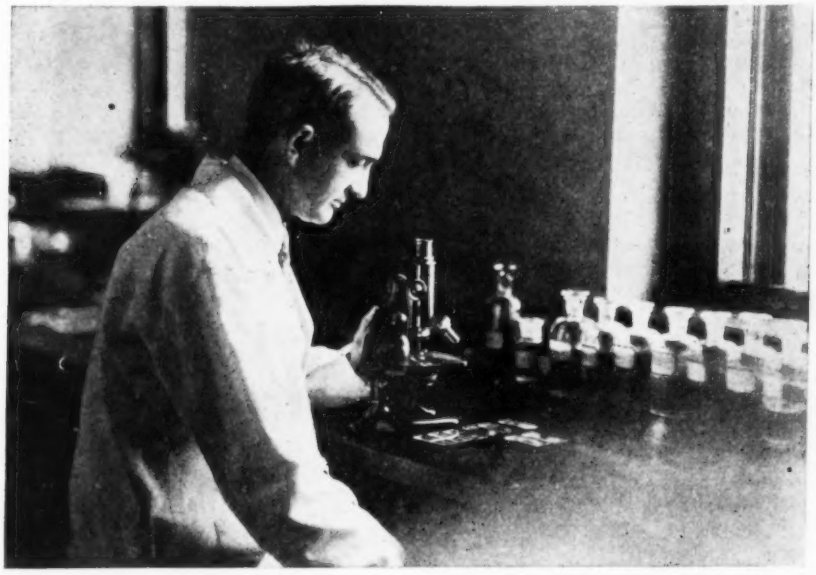
At the Eighth Annual Convention held in Portland, Oregon, in July of this year, the first medal was awarded upon decision of the Research Committee, by the President on the evening of the Annual Banquet to Walter Malcolm Simpson for his original studies on the subject of Tularemia.

Dr. Simpson, a graduate of the University of Michigan, was born December 25, 1895, in Lynn, Massachusetts. At the outbreak of the World War he enlisted as a seaman in the United States Navy and was soon advanced to the rank of Ensign and attached to the U. S. S. Arkansas of the Atlantic Fleet as a watch and gunnery officer. In February, 1919, he returned to the

University of Michigan to complete his premedical studies and received the degree of Bachelor of Science in 1922, Master of Science in Pathology in 1923, and Doctor of Medicine in 1924.

From 1920 to 1923 Dr. Simpson was a teaching assistant in anatomy, histology and pathology in the University of Michigan; from 1923 to 1924 he was instructor in pathology and from 1924 to 1926 was senior instructor in pathology at the University of Michigan. In 1926 to 1927 he was instructor in surgical pathology in Johns Hopkins University and since 1927 has been Director of the Diagnostic Laboratories of the Miami Valley Hospital of Dayton, Ohio.

Dr. Simpson is a Fellow of the American Society of Clinical Pathologists and the American College of Physicians; a member of the American Medical Association, Montgomery County Medical Society, Michigan Academy of Sci-



ence, American Association of Pathologists and Bacteriologists, Ohio Academy of Science and the Central Society for Clinical Research.

At the 1928 meeting of the American Medical Association in Minneapolis Dr. Simpson was awarded a gold medal for his "exhibit of the gross and microscopic changes in tularemia and for excellence of presentation." During the past two years he has contributed nine published papers dealing with the clinicopathologic studies of sixty-four cases of tularemia in and about Dayton, Ohio, and has been engaged with an investigation of the incidence of undulant fever in the same locality. Dr. Simpson's book on Tularemia is just appearing from the press of Paul B. Hoeber, Inc., New York City. His findings in sixty-three cases of undulant fever were also just recently reported before the American Medical Association.

The American Society of Clinical Pathologists takes great pride in numbering among its Fellows men of the caliber of Dr. Walter Malcolm Simpson.

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1929-30

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PERSONALS

Dr. Zera E. Bolin was promoted from Instructor in Pathology to Assistant Professor of Pathology of the University of California on July first.

Dr. Walter E. King has been appointed Assistant Director of the Laboratories and of the Biological Farm of the Parke, Davis and Company, with which he has been connected since 1905.

VALUABLE SUGGESTIONS FOR CONTRIBUTORS TO THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE

"The four rules for the preparation of an article will then be: (1) Have something to say; (2) Say it; (3) Stop as soon as you have said it; (4) Give the paper a proper title."[1]

Let your phraseology express one meaning and one only. Be clear.[2]

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Arrangement.—As authors are quoted in the text give each a number in the order of citation, and number the bibliographic reference with the same number. Arrange the references in a list at the end of the article in the order of the numbers (see below), or arrange items in alphabetical order according to last names of authors, and distinguish between articles by the same author by the use of the date after his name in the text.

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References. (Read these.)

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